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PÁL GYULA

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РЕЗЮМЕ

ИССЛЕДОВАНИЯ РОДА NICOTIANA I. НОВЫЕ РАСТВОРИТЕЛЬНЫЕ СИСТЕМЫ ДЛЯ ВЫДЕЛЕНИЯ АЛКАЛОИДОВ ИЗ NICOTIANA TABACUM L. С ПОМОЩЬЮ ТОНКОСЛОЙНОЙ ХРОМАТОГРАФИИ

Л. ФАРКАШ РИЕДЕЛ

Описываются новые растворительные системы для выделения алкалоидов из обработанных листьев табака методом тонкослойной хроматографии. Хроматограммы тянутся в одном направлении последовательно в одной, двух или трех различных растворительных системах. В вариантах описанного метода расстояние между начальной точкой и фронтом различное в большинстве случаев в последовательных рядах. Эти растворительные системы дают возможность выделить 10—13 компонентов для качественного и полуколичественного анализа. Некоторые компоненты можно даже подвергнуть количественному определению.

ХРОМАТОГРАФИЧЕСКАЯ ГЕТЕРОГЕННОСТЬ МИОЗИНА В КРАСНОЙ И БЕЛОЙ СКЕЛЕТНОЙ МЫШЦЕ КРОЛИКА

Ш. ФАЗЕКАШ, В. СЕКЕШИ-ХЕРМАН, И. КАША, М. САМЕЛ, К. САБО

Статья занимается гетерогенностью миозина в красной и белой скелетной мышце кролика. Найдено, что индивидуальные красные и белые мышцы содержат различные и изменяющиеся подфракции миозина. В общем миозины красной скелетной мышцы характеризовались наличием I и II фракций тогда, когда миозины белой мышцы — наличием фракций III, IV, V и VI. Кроме этого нашли различия во фракциях миозина у индивидуальных мышц потому, что любая из четырех фракций белой мышцы может отсутствовать, даже их пропорция в большой мере меняется. Все подфракции имеют характерную активность ATP-азы, индуцированную Ca^{2+}. Активность ATP-азы и интенсивность флуоресценции в подфракциях красных мышц ниже, чем в подфракциях белых мыщц, и вторичное вещество, видное на хроматограммах, оказалось большим, чем в миозинах белых мышц. Содержание липидов в красных мышцах оказалось на 20—40 процентов выше, чем в белых.

ВЛИЯНИЕ ЦИНКА НА РИБОНУКЛЕАЗНУЮ АКТИВНОСТЬ ВСХОДОВ ОВСА

Й. ВЕТТЕР

Изучали изменение активности рибонуклеазы гомогената листьев после цинковой инкубации. Кроме этого, автор исследовал изменение активности рибонуклеазы всходов овса и кукурузы, между 10-ым и 28-ым днями роста растений. На основе наших результатов можно установить:

1. Инкубация на дестиллированной воде подняла активность рибонуклеазы гомогената листа, но у вариантов, содержавших разные количества цинка (3×10^{-7}–3×10^{-3}М/л) снизила активность.

2. Активность рибонуклеазы контрольного растения уменьшилась в значительной мере только при определенных концентрациях цинка ($3 \times 10^{-5} - 3 \times 10^{-3}$М/л). В этом случае раствор реакции содержал — кроме буфера — фермент, РНК и разные количества цинка.

3. В период роста всходов овса и кукурузы (от 10-го до 28-го дня) активность рибонуклеазы уменьшилась, что свидетельствует о взаимодействии между ростом и рибонуклеазной активностью.

Цинк является одним из важных микроэлементов питания растений и играет многообразную роль в разных физиологических процессах, например в биосинтезе ИУК. Результаты показали, что цинк постоянный компонент нескольких ферментов растительных клеток и отсутствие цинка может вызвать разные патологические изменения в морфологии органов растений, прежде всего в листьях. По нашим данным можно предположить, что цинк оказывает прямое влияние на ферментативные факторы, участвующие в процессах регулирования роста растительных организмов. Регулирование роста является современной, важной проблемой физиологии растений, поэтому надо расширять изучение этой темы. Повидимому, результаты таких исследований можно использовать в практике сельского хозяйства.

СРАВНИТЕЛЬНАЯ ОЦЕНКА ВЫВОДИМОСТИ ПРОСТЫХ И ТРЕХЛИНЕЙНЫХ ГИБРИДОВ ЦЫПЛЯТ МЯСНОГО ТИПА

П. ХОРН

Изучая выводимость трех простых и шести трехлинейных гибридов цыплят в эксперименте, проведенном при случайном распределении в II повторениях установлено, что плодовитость линии от скрещивания белых **Plymouth** в качестве материнской породы с белым **Cornish** в значительной мере превзошла плодовитость матерей, принадлежащих к чистым линиям. Скрещенные матери снесли в среднем на 2,62 процентов больше фертильных яиц, что представляет собой относительное превосходство на 23%. Жизнеспособность в эмбриональном состоянии цыплят трехлинейных гибридов тоже значительно повысилась. 5,64 процентов зародышей у простых гибридов цыплят и 4,89 процентов у трехлинейных гибридов погибли в первые шесть дней выведения. Релятивное превосходство трехлинейных гибридов оказалось 13 процентным. Благодаря лучшей плодовитости и высшей жизнеспособности в ранней эмбриональной фазе выводимость трехлинейных комбинаций, считая на основе количества всех яиц, тоже повысилась в среднем на 2,83 процентов. Когда родительские формы или зародыши, развивающиеся в яйцах подвергались влиянию неблагоприятных условий внешней среды, трехлинейные гибриды еще в большей степени превосходили простые гибриды с точки зрения и эмбриональной смертности, и выводимости, что высчитано в процентах от количества яиц, положенных в инкубаторе. В противовес обычным тенденциям несколько комбинаций показали высшую степень и достоверный гетерозис. Среди реципрокных с материнской стороны комбинаций не было обнаружено значительного различия.

ДАННЫЕ К ПОЗНАНИЮ ДИАПАУЗЫ *Hyphantria cunea Drury (Lepid.: Arctiidae)* В ВЕНГРИИ

ДЬ. ШАРИНГЕР

Диапауза **H. cunea**, проявляющаяся в кукольном состоянии при 18 и 23°C, определяется в первую очередь фотопериодом. Более высокая температура (28°C) уменьшает влияние фотопериода. Критическое время освещения, вызывающее диапаузу, находится в пределах 14 и 15 часов. Качество растения, используемого в качестве питания тоже в какой-то мере обуславливает диапаузу. Покой, вызванный фотопериодом не изменился под влиянием меняющейся температуры (под открытым небом, в 1970-м году). Личинки воспринимают условия освещения во второй части развития. Величина популяции бабочек второго летнего, и третьего, неполного поколения (личинок) зависит в большой мере от температуры. В годы с высокой средней температурой ущерб больше.

ИСПОЛЬЗОВАНИЕ ЛИСТОВОЙ МУКИ И СЕНА СОИ И ПОДСОЛНЕЧНИКА В ОТКОРМЕ МЯСНЫХ СВИНЕЙ

Э. КУРНИК, Д. ГАБОШ, Б. И. ПОЖАР

Откормочный опыт производился на трех группах больших белых мясных свиней типа bacon, чтобы экспериментально оценить биологическую эффективность сенной и листовой муки подсолнечника и сои. Для откорма в каждой группе использовали по 15 голов молодняка. Первые две группы откармливались по 70 дней, а третья 50 дней. После 1—2 дневного отвращения все животные ели корм, дополненный смесью листовой и сенной муки подсолнечника в пропорции 1 : 1, а подобную смесь сенной и листовой муки сои ели сразу и охотно. Из сопоставления порций выявлено, что свиньи съедали без остатка корм, составляющий 5% их веса. На основе производственных данных оказалось, что хотя мука подсолнечника и сои по содержанию белка не рассматривается как обогащенная, содержание белка муки заменило белок корма в случае кормового ячменя. Ежедневное среднее увеличение привеса трех групп — 630 г., от него только немного отстает среднее увеличение привеса у свиней, скормленных мукой подсолнечника (622 г.) в то время, когда контроль (634 г.) был превышен при скармливании мукой сои со средним 668 г. Этим также доказано, что белок сои обеспечивает большую биологическую эффективность именно его отличным аминокислотным спектром. Живой вес молодняка в опыте достиг 65—79 кг. в течение 4 или 4,5 месяцев. Из данных по увеличению привеса выявлено, что суточное увеличение привеса животных колеблется в относительно широких пределах, причины этого явления до сих пор неизвестны.

ФАКТОРЫ, ВЛИЯЮЩИЕ НА КОЛИЧЕСТВЕННЫЕ АНАТОМИЧЕСКИЕ ПРИЗНАКИ ВИНОГРАДНОЙ ЛОЗЫ, II. СТРУКТУРА МЕЖДОУЗЛИЙ НА РАЗНЫХ УРОВНЯХ ЛОЗЫ

А. ХЕГЕДЮШ

Изучались количественные анатомические признаки поперечных сечений, вырезанных из середины каждого междоузлия зрелой виноградной лозы. Проводились те же самые измерения, вычисления и калькуляция отношений, которые были опубликованы раньше (ХЕГЕДЮШ 1967). От одного междоузлия до другого анализировалась флуктуация значений, а также их повышающаяся или понижающаяся природа вдоль лозы. В значительной части количественных анатомических признаков определенная увеличивающаяся или уменьшающаяся тенденция наблюдалась вдоль лозы от основания до вершины, поэтому если мы хотим показать различия, вызванные сортом или экологическими эффектами, необходимо выбрать образцы с идентичного или почти идентичного уровня. Что касается положения изученного междоузлия по отношению к усикам, не было найдено различия в структурных индексах.

ИЗУЧЕНИЕ ОТНОШЕНИЯ МЕЖДУ СОДЕРЖАНИЕМ ЦИНКА В СЕМЕНАХ И ГРУППОЙ СОЗРЕВАНИЯ ГИБРИДОВ КУКУРУЗЫ

ДЬ. ДЁРИ, М. ПАЛКОВИЧ

Концентрация цинка в гибридах кукурузы меняется в зависимости от группы созревания. В позднеспелых гибридах концентрация цинка понизилась и в эндосперме, и в зародыше + перикарпий у 17 образцов, за исключением двух гибридов.

ВЛИЯНИЕ ДОЗЫ УДОБРЕНИЯ И ОРОШЕНИЯ НА ЗАРАЖЕНИЕ ФУЗАРИОЗОМ ОЗИМОЙ ПШЕНИЦЫ

Э. БОЦ, Эл-С. ХЕФНИ, О. ТОТ

Болезни фузариоза пшеницы исследовались во всем мире в течение нескольких десятков лет. В этом опыте благодаря фузариозу зерна сморщились, абсолютный вес и процент всхожести понизились при высокой степени азотного удобрения. Наивысший средний процент всхожести на неорошенных делянках составил 88, в то время как самое низкое среднее равное 82,5 наблюдалось при самой высокой дозе удобрения. На орошенной делянке наивысший процент всхожести контроля был 90%, а самый низкий процент — 81, при самой высокой дозе удобрения. Хлебопекарное качество постепенно улучшалось с повышением дозы азотного удобрения до 300 N кг/га без орошения в то время как оно достигло пика при удобрении 200 N + 100 P_2O_5 + 100 K_2O кг/га с орошением.

ВЛИЯНИЕ МЕТОДОВ КАСТРАЦИИ И ИЗОЛЯЦИИ, ВРЕМЕНИ ОПЫЛЕНИЯ И ПРОИСХОЖДЕНИЯ ПЫЛЬЦЫ НА ЗАВЯЗЫВАНИЕ ПЛОДОВ У ГРУШИ

Й. НИЕКИ

Влияние методов кастрации и изоляции, времени опыления и происхождения пыльцы на завязывание плодов у груши изучалось в 1968—1970 гг. Полная кастрация была точно так же действенной как и удаление пыльников. Это наиболее возможно, быстро и надежно в практике, чем удаление пыльников. Из методов изоляции, применяемые пергаментные изоляторы оказались лучшими, в целофановых изоляторах кастрированные цветки высыхали. Изоляция в сетчатых изоляторах была непригодной для исключения возможности перекрестного опыления. Оптимальное время опыления варьировало в соответствии со стадиями цветения и сортом. Наибольший процент завязывания плодов был получен при опылении, проведенном во время полного цветения, и в стадию раскрытия бутонов. Между собранной пыльцой и полученной прямо из изолятора не были обнаружены достоверные различия по фертильности и размеру образованных пыльцевых трубок.

ДЕЙСТВИЕ ГИДРАЗИДА МАЛЕИНОВОЙ КИСЛОТЫ НА ПОЛУЗРЕЛЫЕ ПЛОДЫ АБРИКОСА И СЛИВЫ, А ТАКЖЕ ЕГО РЕВЕРСИЯ В АСКОРБИНОВУЮ КИСЛОТУ

Д. ШУРАНИ

Изучалось отдельное и совместное влияние гидразида малеиновой кислоты и витамина С на полузрелые плоды Венгерского абрикоса С. 235, Краснощёкого абрикоса С.778 и сливы Бестерцеи. Во время хранения самая маленькая потеря наблюдалась после обработки 2000 ppm ГМ, но концентрация 500 ppm тоже оказалась хорошей. Уже 1000 ppm витамина С значительно способствовал потере веса плодов. На основе результатов опытов найдена положительная корреляция между содержанием сухого материала и потерей веса при хранении тогда, как отрицательная корреляция получена между отношением инвертного сахара к кислоте и потерей веса при хранении. У плодов с большим отношением инвертного сахара к кислоте наблюдался самый маленький ущерб. Синергизм получился благодаря комбинированной обработке с 500 ppm ГМ, которая по эффективности оказалась эквивалентной самому сильному действию аскорбиновой кислоты. На основании этого автор утверждает, что результативность обработок гидразидом малеиновой кислоты зависит от биологического уровня витамина С, присутствующего в плодах во время опрыскивания.

GYULA MÉSZÖLY
1910—1974

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GÁBOR UBRIZSY

1919—1973

I — once a professor and always a true friend of Gábor Ubrizsy, the outstanding Hungarian scholar of plant protection, mycology and geobotany — received the news about his unexpected death with the same sorrow and astonishment as did all the other Hungarian experts of these sciences.

Gábor Ubrizsy was born on 23 September 1919 at Ungvár. His father was a petty clerk in county Szabolcs, who had great difficulties in raising his six children. Ubrizsy spent the years of his childhood and youth at Nyíregyháza, and it was there too that he completed his secondary school studies with excellent results in 1937. At the Debrecen University he studied natural history and geography for five years, passed his examinations always with excellent results, and was granted a teacher's diploma in 1942. As an unpaid practitioner he took part in floristical, coenological and mycological researches at the botanical institute even in the second year of his university studies. His first paper — the attempt of an autodidact — was published in the "Szabolcsi Szemle" (Review of County Szabolcs) when he was only seventeen years old, and from 1939 valuable data were presented by him on the fungi and flowering plants of the Nyírség.* Of his extensive work on the vegetation of Mountain Vihorlát only minor details were published (1941). It was again quite early that he started thorough studies on phytopathology, and in June 1941 was appointed assistant at the Agricultural College of Debrecen-Pallag. At the end of the same year he passed his examination for a doctor's degree before

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* Region in the north-eastern part of Hungary.

Imre Máthé, temporary head of the department of botany. His dissertation written on the mycoflora of Nyírség was published in a final form in the *Acta Geobotanica Hungarica* at Kolozsvár* (1943). After a short time spent at Kolozsvár he began to work in the field of agricultural professional education as an agricultural secondary school teacher, in Debrecen, until July 1946 — with a break of two years spent in military service and captivity — then at Szarvas up to March 1949. For a year he worked in the Agricultural Scientific Centre, and on behalf of the government concluded an agreement on plant sanitation with the Czechoslovakian People's Republic, then reorganized the Institute of Plant Sanitation under the name Research Institute of Plant Protection where he became director and scientific leader from March 1950 to 1959. During this period he organized the yearly repeated National Conferences on Plant Protection, gave reports at international congresses on plant protection (1951—1970: Sofia, Warsaw, Peking, Moscow, Vienna, Paris), in committees elaborating control measures against potato cancer and the American white coddling moth; in 1952 assisted in organizing the control of the olive-tree fly, etc. He delivered numerous lectures at the Hungarian Academy of Sciences, and in Hungarian and foreign scientific societies. He played an important role in elaborating agricultural development plans, and organizing and directing research work in plant protection and botany at various posts (as president of the Committee on Plant Protection and Agricultural Work Committee on Environment Protection of the Hungarian Academy of Sciences and a member for years of its Botanical Committee; a member of the presidency of the Hungarian Association of Agricultural Sciences and president of its Society of Plant Protection; head of the work committee on environment protection of the OMFB; during their existence he was president of the 37th co-ordinating committee, and member of the 51st one, etc.), and was member of the presidency or consultant of a number of international organizations on plant protection (European Plant Protection Organization, Centre International des Antiparasitaires, European Weed Research Council). His activity was rewarded in 1951 with the silver medal of Kossuth Prize, and in 1964 with the golden medal of the Order of Labour, and was given the golden diploma of the MAE too. In 1952 he was awarded the title of doctor of biological sciences. The Hungarian Academy of Sciences elected him in 1965 a corresponding, and in 1973 a regular member of the section of agricultural sciences as if disapproving of the undeserved neglection which was his part in the last years of his life. In 1969 he was dismissed as leader of the Institute (and remained its scientific consultant until his death). As for his university activities it must be mentioned here that in April 1949 he was appointed private docent of mycology at the Debrecen University and taught this subject there for two years, then

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* Town in Transylvania, at present a part of Roumania.

for a long time at the Budapest University. In 1964 he was given the title of university professor at the University of Horticulture which he resigned not long before his death. Alas, in his serious illness recognized a few weeks earlier he only could rejoice his being appointed regular member of the Hungarian Academy of Sciences for a couple of days. He died of pulmonary embolism on 25 May 1973, at the age of 53. At his funeral on 2 June speeches were delivered by the author of this paper, by Zoltán Király corresponding member of the Academy and by his successor: Tibor Jermy. The words written in his necrologue: "With the departed we have lost a great expert of general plant protection phytopathological mycology, herbology and environment protection who by reorganizing the Research Institute of Plant Protection has ensured international fame and level for phytopathological research in Hungary" — are one by one true but not complete. Gábor Ubrizsy was, namely, an outstanding research worker in more than one branch of botany. After the death of our famous mycologist Gusztáv Moesz (1946) Ubrizsy was a leading representative of this science too in Hungary. It was he who prepared Moesz' posthumus work for the press, and contributed to the knowledge of not only the Hungarian fungi with numerous particulars. Unfortunately, his synthesis of the Hungarian fungi started in 1967 (Review of mycoflora of Hungary) could not be completed. He was co-author of the identification handbook of Hungarian mushrooms (1951) and Hungarian macrofungi (1953) edited by the Hungarian Academy of Sciences, and a collective work, the handbook of Hungarian microfungi, in the compilation of which he played a leading role, is expected to be published soon. In the author's university text-book (Fejlődéstörténeti Növényrendszertan [Phylogenetic Phylotaxonomy], 1.—3. editions, 1953—1965) the sections on mycology were written by Ubrizsy *(Myxophyta, Mycophyta)*. It was after that that he built up his own new evolutionary mycosystem (with József Vörös, 1964, 1966) which, however, was not accepted due to its complexity and irregular nomenclature. (Ubrizsy was right in eliminating e.g. the former class of heterogeneous *Archimycetes*, and including the slime fungi (earlier a separate division [phylum]: *Myxophyta*) in the division of *Mycophyta* (in Ubrizsy's nomenclature: "*Mycota*") fungi as a subdivision; but the use of certain names (e.g. *Mycomycotina*) and collective orders between subdivisions and classes — like the "Überordnung"-s of Takhtajan and Ehrendorfer, or lichens placed among *Ascomycetes* and *Basidiomycetes* (Cronquist 1960) — found in some mycosystems after 1960 was incorrect, and later — quite rightly — he gave it up too (cf. Soó Bot. Közl., *52*, 50—51, 1966). Recent views of the taxonomy of fungi see e. g. Kreisel 1969, Mädgefrau in "Strassburger Lehrbuch" 1971.*

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* In his book Grundzüge eines natürlichen Systems der Pilze (Jena 1969) Kreisel excludes again from the strain of fungi (in his book *Eumycota*) not only the slime fungi placed in 3 independent groups but also the *Oomycetes* ranked with *Phycomycetes*, which he derives

As a disciple of the school of plant coenology in Debrecen Ubrizsy was the first to start mycocoenological investigations in Hungary (see his above mentioned doctor's dissertation and subsequent studies on the coenological conditions of macrofungi in different types of forest, 1957, 1966, 1972) with remarkable results, as did Gábor Bohus who himself was excellent in this field as well. Ubrizsy was similarly interested in the weed plant communities and prepared numerous analyses of Hungarian plant associations. He dealt with

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from the *Chrysophyta* and regards them as one of their classes. (This group was considered even by Gäumann to originate from the order of *Siphonales* (*Chlorophyta*) in contrast with the other fungi traced back to *Flagellatae*; with Ubrizsy and Vörös it is *Peronosporales* of which the origin is uncertain.) Kreisel, on the other hand, restores in a wider sense the *Archimycetes*, as the class of *Chytridiomycetes* Cejp, including the more primitive *Oomycetes*, but excludes from the range of "*Eumycota*" the *Plasmodiophorales* (placed earlier among *Archimycetes*, and by Ubrizsy—Vörös among lime fungi). Kreisel distinguishes 5 classes (*Endomycetes*, *Zygomycetes*, *Asco-* and *Basidiomycetes* in addition) and 63 orders. It is remarkable that he places *Ustilaginales* among *Endomycetes*. Ubrizsy's system is in many respects more conservative, the number of orders outside the slime fungi is 51.

The most recent edition of the Bonn text-book (Mägdefrau 1971) uses — certainly for didactic reasons — a simpler and easier to survey mycosystem. All fungi (*Mycophyta* strain) are grouped in 4 classes: *Myxomycetes*, *Phycomycetes* (including the earlier *Archimycetes*, the much discussed *Plasmodiophorae*, and the classical *Oomycetes* and *Zygomycetes* as "orders"), *Ascomycetes* and *Basidiomycetes*. On the other hand, the class or *Ascomycetes* is broken down to 5 sub-classes (instead of the former two classes; *Proto-* or *Hemi-* and *Euascomycetidae*, with Kreisel the former one is a separate class; *Endomycetes*, while latter, the *Ascomycetes*, has two further subclasses; *Euascomycetidae* (in a different interpretation most *Ascomycetes*) and *Loculoascomycetidae*, as *Myriangiales*, *Dothideales*, *Hemisphaeriales*, *Hysteriales*, etc.). The classes of Mägdefrau are, on the other hand; *Protascomycetidae* (*Endomycetales*, *Taphrinales*), *Plectomycetidae* (*Plectascales*, *Erysiphales*), *Loculomycetidae* (*Myriangiales*, *Dothiorales*, *Pseudosphaeriales*), *Pyrenomycetidae* (*Sphaeriales*, *Clavicipitales*, *Laboulbeniales*), *Discomycetidae* (*Pezizales*, *Helotiales*, *Phacidiales*, *Tuberales*); this way of classification is more easily compared with the systems of Gäumann, Werdermann (in Engler Syllabus I. ed. 1954), Ubrizsy (in Soó 1953—1965), Ubrizsy—Vörös 1964, 1965. In all these systems *Basidiomycetes* are divided into 2 sub-classes; *Holo-* or *Homobasidiomycetidae*, fungi of undivided basidia generally considered older (except by Mägdefrau), and *Phragmobasidiomycetidae*, fungi of divided basidia.

Kreisel goes farther here too, by placing a large proportion of *Ustilaginaceae* from the latter sub-class among *Endomycetes*; he sets up, otherwise, 2 further sub-classes; *Gasteromycetidae* (the former *Gasteromycetes*), *Hymenomycetidae* (former *Hymenomycetes*), but with some orders he cannot do anything (e.g. *Exobasidiales*, *Tilletiales*), and enumerates them as a "Restgruppe". It is surprising too that he classifies the "Fungi imperfecti" or *Deuteromycetes*, the fungi only known in a conidial form, as *Ascomycetes* and *Basidiomycetes* imperfecti which is an unsatisfactory solution.

The most remarkable is, however, the evaluation of lichens. From an evolutionary point of view they are undoubtedly a secondary form, but their shape is determined primarily by the fungus component. It was for this reason that Ubrizsy — like myself — regarded them as a class of fungi. In contrast to this Kreisel classes the lichens among *Ascomycetes*, *Basidiolichenes* are — namely — completely missing from his system, and he cannot evaluate the only "phycolichene" *Geosiphon*. According to the other, more general, extreme view the lichens are considered to be a separate division (e.g. Engler Syllabus, the Bonn text-book, etc.), which is only justified didactically.

Ubrizsy and Vörös gave a clear summary on the phylogenesis of fungi in 1970—71 which, however, did not contain anything new, though they cited new authors as Ainsworth, Arx.

These comparisons too suggest that the system and phylogenesis of fungi have not been perfectly cleared up yet, and even the best mycologists' opinions are contradictory just as in the case of the flowering plants (cf. Soó 1967 Acta Bot. Hung., 1973 Syn. florae veget. Hung. V).

the coenological, synecological and dynamical conditions of rice fields (1948, 1961), vineyards (1967) and waste lands in the first place, but also of sowing- and ruderal associations, mostly in connection with their economic importance and with chemical weed control, first of all with the effect of the latter on the transformation of agrophytocoenoses (a number of papers between 1948 and 1972). In his last years he was the greatest expert in weed coenology. He mentioned only a few months ago that he planned to write a detailed synthesis of Hungarian weed associations; it is a great loss of Hungarian geobotany that it could not be realized. He earlier performed experiments too, to study the conditions of artificial associations, in which — as later he himself admitted — he overestimated allelopathy as a coenological factor (1942—43). He also carried on ecological studies of different character with macrofungi (e.g. mycoflora and R-factor, 1948). He was, otherwise, the reader of the fourth and fifth volumes of the author's main work: Handbook of the Hungarian flora and vegetation . . ., and contributed valuable information to it; the author expresses his thanks for his unselfish assistance in this place too.

His research work in plant protection was extremely diversified; he was the founder of "integral plant protection" in Hungary. He discussed the results and disadvantages of chemical weed control in many books and scientific and popular papers. He pointed out the changes occurring in the flora in response to herbicide rotation and continuous herbicide treatments, the harmful consequences for, and negative reactions of the biosphere. These were summarized by him in his book "Vegyszeres gyomirtás" (Chemical weed control) (1958, 1962) and in a very successful booklet published in the series: "Korunk tudománya" (Science of our age): "Peszticidek, áldás és átok" (Pesticides, blessing and plague) (1969).

Gábor Ubrizsy was the editor and co-worker of many hand-books on plant protection and practical mycology. Leaving the rather popular guides and practical publications written for farmers or gardeners unmentioned we have to emphasize here—in addition to those spoken of before — the following works: "A növényvédelem gyakorlati kézikönyve" (Practical handbook on plant protection) published in three editions (1951—1960) — its first edition brought him the Kossuth Prize; "Termesztett növényeink védelme" (Protection of cultivated plants in Hungary) (1958) written with G. Reichart as co-worker; "Növénykórtan" (Phytopathology) (1952, 1965) and "Mezőgazdasági mykologia" (Agricultural mycology) (1968) written with J. Vörös as co-worker and awarded a prize by the Publishing House of the Hungarian Academy of Sciences. In the former Ubrizsy wrote up the history, basic concepts and scope of subjects of phytopathology as well as the biological control, it was here that he used his new system of fungi too. Sections on biology, biochemistry and genetics prove that phytopathology is no longer a mere descriptive and practical science (cf. Soó: Bot. Közl. loc. cit.). Yet, from the work running to more than 1500 pages the rudiments and methodics of mycology as well as a detailed key of identification were omitted from the section on fungal diseases. These are discussed in an enlarged and revised form, together with the micromorphology, taxonomy, evolution and physiology of fungi, in the "Mezőgazdasági mykologia" (Agricultural mycology). The taxonomic keys summarized in 180 tables — besides covering all fungi causing plant diseases — take into account the saprophyte species too, and even those important from industrial and medicinal points of view (cf. Bot. Közl. *55*, 125, 190). In his work "Magyarország kultúrflórája" (Cultivated plants of Hungary) (1956) written with J. Vörös as co-author he wrote up the mould fungi, and wrote numerous headwords in the "Növényvédelmi Enciklopédia" (Encyclopedy of Plant Protection) too (1968).

Ubrizsy's other editorial activities are also highly valuable. He took part in the editing work of various scientific reviews ("Magyar Gombászati Lapok" [a mycological publication] [1944—48]; "Mezőgazdasági Tudományos Közlemények" [an agricultural journal]; *Acta Agronomica Academiae Scientiarum Hungaricae*, Publications of Section IV. of the Hungarian Academy of Sciences), and he was, of course, the editor of the Year-book of the Research Institute of Plant Protection until quite recently. He initiated the publication and was general editor until his death of the phytopathological review of the Academy of Sciences: *Acta Phytopathologica Academiae Scientiarum Hungaricae*, first published in 1966.

Ubrizsy was author or co-author of some 20 books, the number of his scientific papers was some 140, while the popular educational publications and reports written by him even exceeded the number of 240. He performed, namely, an extensive work in propagating general knowledge (Society for the Propagation of Natural Sciences, Association of Agricultural Sciences, Patriotic People's Front, etc.).

Ubrizsy was, however, not only a scholar in botany, plant- and environment protection, but was also highly educated in the history of art and literature. While most natural scientists are not interested in anything outside their special lines, Ubrizsy was one of the most outstanding art-collectors. His collection of paintings and statues gives a full picture of Hungarian art in the 19th and 20th centuries, from Károly Markó and István Ferenczy to the modern abstract and surrealist artists including almost all great Hungarian masters.

I was attached to my dear old pupil Gábor Ubrizsy not only through the love of nature, science and art, but also by the bonds of the truest friendship. His memory will be kept by his friends, co-workers, pupils and by the Hungarian sciences of botany and agriculture.

R. Soó

GÁBOR UBRIZSY'S MAIN WORKS

1. Books

1951, (1953, 1960) A növényvédelem gyakorlati kézikönyve* (Practical hand-book on plant protection). (Berend, I., Csorba, Z., Fehérváry, G., Husz, B., Jermy, T., Kerekes, L., Manninger, G. A., Molnár, L., Podradszky, J., Reichardt, G., Sántha, L., Szelényi, G., Terényi, S., Ubrizsy, G. Ed. Ubrizsy, G.) Mezőgazdasági Kiadó, Budapest, 580, I—II, 854, I—III, 831.

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1952, (1955) Növénykórtan* (Phytopathology). (Berend, I., Csorba, Z., Dohy, J., Husz, B., Király, Z., Klement, Z., Milinko, I., Podradszky, J., Sántha, L., Szatala, Ö., Szirmai, J., Ubrizsy, G. Ed. Ubrizsy, G.) Akadémiai Kiadó, Budapest, 1076, I—II, 1200.

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1958, (1962) Vegyszeres gyomirtás (Chemical weed control). (Ubrizsy, G.) Mezőgazdasági Kiadó, Budapest, 252, 428.

Termesztett növényeink védelme* (Protection of cultivated plants in Hungary). (Ubrizsy, G., Reichardt, G.) Mezőgazdasági Kiadó, Budapest, 447.

1960, A penészgombák, *Mucorales*, *Hyphomycetes** (Mould fungi, *Mucorales*, *Hyphomycetes*). (Ubrizsy, G., Vörös, J.)

Magyarország kultúrflórája (Cultivated plants in Hungary). Akadémiai Kiadó, Budapest, 124.

1968, Mezőgazdasági mykológia* (Agricultural mycology). (Ubrizsy, G., Vörös, J.) Akadémiai Kiadó, Budapest, 576.

1969, Peszticidek, áldás és átok (Pesticides, blessing and plague). (Ubrizsy, G.) Akadémiai Kiadó, Budapest, 113.

A vegyszeres gyomirtás gyakorlata* (Practice of chemical weed control). (Ubrizsy, G., Gimesi, A.) Mezőgazdasági Kiadó, Budapest, 310.

2. Study series

1941, A Nyírség gombavegetációja (The fungi of Nyírség). Tisia, *5*, 1—51.

1943, Szociológiai vizsgálatok a Nyírség gombavegetációján (Sociological investigations on the fungal vegetation of Nyírség). Acta Geobot. Hung., *5*, 251—279.

1948, A rizs hazai gyomnövényzete (Weed plants of rice in Hungary). Acta Agrobot., *1*, 1—44.

1961, Unkrautvegetation der Reiskulturen in Ungarn. Acta Bot. Acad. Sci. Hung., *7*, 175—220.

1950, Magyarország ruderális gyomnövényszövetkezetei, tekintettel a mezőgazdasági vonatkozásokra (Ruderal weed plant associations of Hungary and the agricultural aspects of the problem). Mezőgazd. Tud. Közl., *1*, 87—118.

Les associations de mauvaises herbes rudérales de la Hongrie et les aspects agricoles du problème. Acta Agron. Acad. Sci. Hung., *1*, 107—159.

1955, Die ruderalen Unkrautgesellschaften Ungarns. Studien über Ökologie und Sukzession. Acta Agron. Acad. Sci. Hung., *5*, 393—418.

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1970, Recherches de base agrophytocénologiques et le désherbage. Acta Phytopath., *5*, 341—354.

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1972, Nouvelles analyses mycocénologiques dans certains types sylvicoles de la Hongrie. Acta Phytopath., *7*, 101—130.

1966, A new conception in the review on the phylogeny and system of Fungi.* (Ubrizsy, G., Vörös, J.) Acta Bot. Acad. Sci. Hung., *12*, 199—220.
1970. (1971) A gombák törzsfejlődése és rendszere (The phylogeny and system of Fungi). Mikol. Közl., *12*, 51—54, *13*, 3—12.
1967, Review of Mycoflora of Hungary. I. Phycomycetes. Acta Phytopath., *2*, 153—178.
Review of Mycoflora of Hungary. II. Protomycetales. Acta Phytopath., *2*, 267—285.
Review of Mycoflora of Hungary. III. Ustilaginales. Acta Phytopath., *2*, 389—411.
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Review of Mycoflora of Hungary. Addition to Part I. Acta Phytopath., *3*, 431—458.

*with co-authors

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STUDIES ON THE GENUS NICOTIANA I.

NEW SOLVENT SYSTEMS FOR THE SEPARATION OF ALKALOIDS FROM NICOTIANA TABACUM L. BY THIN-LAYER CHROMATOGRAPHY

By

L. FARKAS-RIEDEL

DEPARTMENT OF APPLIED BOTANY AND HISTOGENESIS, L. EÖTVÖS UNIVERSITY, BUDAPEST

New solvent systems are described for the separation of tobacco alkaloids from cured tobacco leaves by thin-layer chromatography. The chromatograms are run in one direction, in one, two or three different solvent systems consecutively. In the variants of the method described, the distance between the starting point and front is different, in most cases, in the consecutive runs. These solvent systems make a separation of 10—13 components for both qualitative and semiquantitative assays possible. Some components can even be subjected to quantitative determination.

Introduction

Tobacco breeding, as well as biochemical studies on alkaloid biosynthesis (e.g. the investigation of demethylation of nicotine into nornicotine) make it necessary to develop sensitive analytical techniques for the separation and quantitative assay of individual alkaloids. Although the qualitative and semiquantitative paper chromatographic methods of PORTER—NAGHSKI—EISNER (1949), WERLE—KOCH (1951), KRAFT (1953), LEISERSON—WALKER (1955), JEFFREY—TSO (1955) used also by WADA (1956), STEPKA—DEWEY (1961), PŁATEK (1964a, 1964b), separate most alkaloids and their derivatives, because of the long time needed to run the chromatograms (1 to 2 days), and because of the relatively low sensitivity of the procedures, they are not suitable for the separation of tobacco alkaloids. Therefore, a number of authors developed more sensitive thin-layer chromatographic (TLC) techniques (PAPP—SZABÓ 1963, SPEAKE—MCCLOSKEY—SMITH—SCOTT—HUSSEY 1964, FEJÉR-KOSSEY 1964, 1967, HODGSON—SMITH—GUTHRIE 1965, WINEFORDNER—MOYE 1965, LEETE 1968). However, most of these methods are suitable for the separation of a few alkaloids only (PAPP—SZABÓ 1963, SPEAKE—MCCLOSKEY—SMITH—SCOTT—HUSSEY 1964, WINEFORDNER—MOYE 1965, LEETE 1968). Alternatively, as the methods are two-dimensional (HODGSON—SMITH—GUTHRIE 1965, FEJÉR-KOSSEY 1967), they are time-consuming and expensive.

A summary of the results obtained by various authors is presented in Tables 1/A and 1/B. The thin-layer chromatographic separation methods

Table 1/A

*R_f-values × 100 of the tobacco alkaloids and related compounds, as obtained by various chromatographic methods (paper chromatography, one- and two-dimensional thin-layer as well as circular paper chromatography)**

Alkaloids and related compounds	Paper chromatography												Thin-layer chromatography									
	1-dimensional								circular				1-dimensional						2-dimensional			
	** A				B	C	D		E				F	G	H	I	J	K	L		M	
	I	II	III	IV			I	II	I	II	III	IV							I	II	I	II
N-methylmyosmine	20	17	26	28	—	13	31	28														
2-hydroxynicotine	20	18	26	38																		
nornicotine	28	26	32	49	42	17	41	41	64	55	55	41	30	—	18	26	15	25 31	34	5	27	5
anabasine	31	32	39	66	50	21	55	41	76	63	70	53	44	—	27	48	50	35	50	6	44	7
3-(4-aminobutyl)-pyridine	37	32	30	29	52																	
dihydrometanicotine	37	34	35	24																		
m-nicotine	40	36	35	41	4	25	62	55											—	—	14	4
nicotine	43	49	80	79	45	33	75	75	92	77	97	86	57	48	50	80	82	45 50	77	8	73	14
dihydronicotyrine	51	57	85	87	—	36	—	—														
myosmine	85	87	86	68	—	92	—	—											—	—	74	23
nornicotyrine	91	90	87	82	—	97	—	—														
nicotyrine	91	91	92	85	92	98	93	84	—	—	80	—						75 80	87	92	81	76
nicotinamide	—	—	—	—	70	86	64	59														
anatabine	—	—	—	—	47	—	—	—										41 44	—	—	57	7
oxynicotine = nicotine N-oxyd						30	36	33										0	8	5	6	2
N-methylanabasine						36																
2,3'-dipyridyl = isonicoteine						95												60 70	—	—	73	40
isonicotinic acid						51																
nicotinic acid						62	15	12														
2-methyl-6(3-pyridyl)-tetrahydro-1,2-oxazin						96	90	85														
3-acetylpyridine						61																
-picoline						87																
cotinine																			75	76	—	—
norcotinine																			50	51	—	—
nicotone																			—	—	74	28
4-methylamino-1(3-pyridyl)-1-butanol						12																

* Compiled from literary data.

** Letters refer to various authors and the separation techniques used by them (cf. Table 1/B).

summarized in Table 1/A were found nowadays to be unsatisfactory. I have attempted to elaborate one-dimensional procedures, by using several solvent systems consecutively to develop the same plate. The choice of one-dimensional instead of two-dimensional chromatography is explained by the fact, that the method was developed to follow the nicotine → nornicotine demethylation step quantitatively in tobacco leaves. These alkaloides are non-fluorescent and reagents are necessary for their detection. In the case of one dimensional chromatography, the sample can be placed in spots or lines, as shown in Fig. 1, in several replications on the same plate. Under the same conditions, on this plate the equal substances run to about the same distance (Fig. 1). Therefore, a part of the chromatogram can be treated with reagents and untreated areas cor-

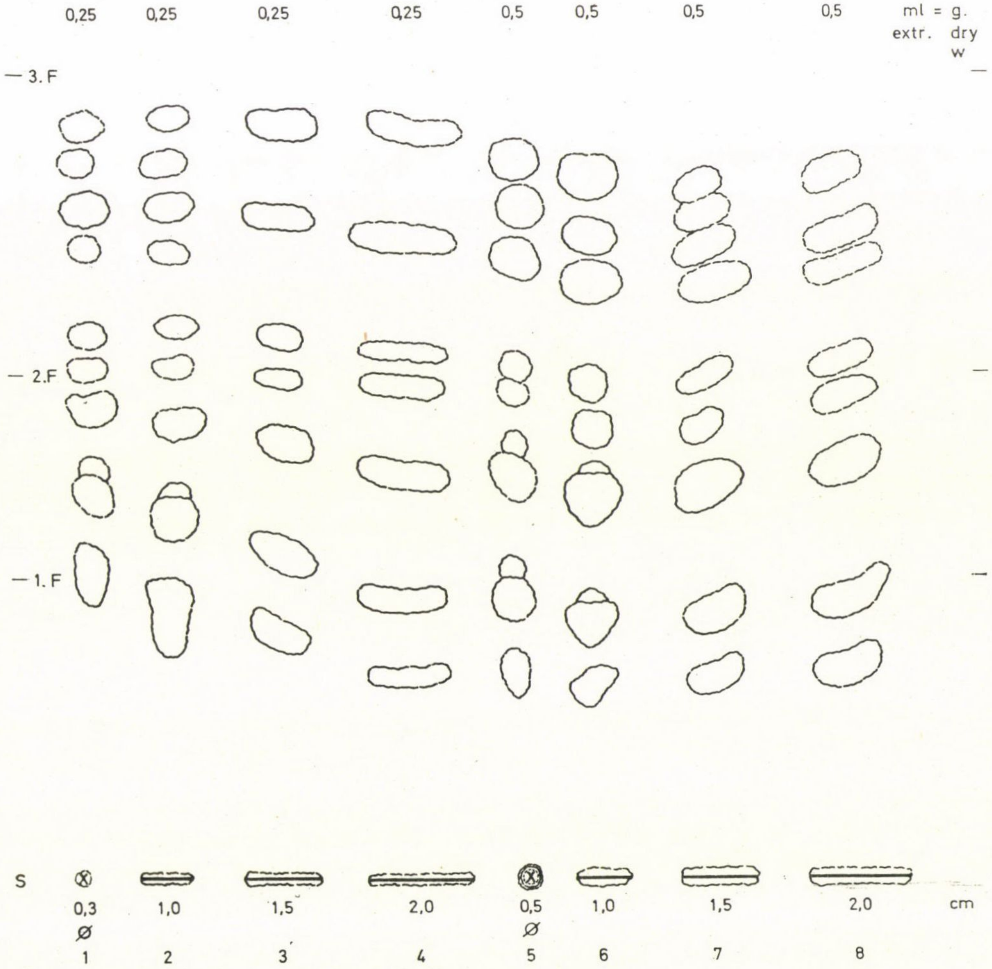

Fig. 1a

Table 1/B

Details of techniques used by various authors for the separation of tobacco alkaloids
Supplement to Table 1/A

Author	Paper or thin-layer	Preparation of				Detection
		extract or authentic substances	stationary phase	vapour atmosphere used for	mobile phase (solvent system)	
		with				
A) PORTER—NAGHSKI—EISNER (1949)	Whatman No. 1	—	—	—	I. n-butanol-acetate buffer* pH 5.6 II. n-butanol-benzene-buffer* 85 : 5 : 30 III. methanol-n-pentanol-benzene-buffer* 31 : 15 : 50 : 8 IV. butylacetate-methanol-25%aq. NH_3 95 : 5 : 25	1% iodine in 95% ethanol, brown colouration
B) WERLE—KOCH (1951)	Schleicher-Schüll 2043/b or Whatman No. 4	n HCl to pH 7	—	—	butanol-10% acetic acid gl.-20% water	CNBr vapour, spray of 2% sol. of aniline in M/15 phosphate buffer or a 0.25% sol. of benzidine in 50% aq. ethanol, different colouration
C) TSO—JEFFREY (1953)	Whatman No. 1	—	—	water	tert. amyl-alcohol-acetate buffer* pH 5.6 50 : 50	*a)* KI—Pt solution *b)* 1% β-naphthylamine in ethanol, then CNBr vapour *c)* PABA in ethanol, 1% then CNBr vapour

D) Leiserson—Walker (1955)	Whatman No. 1	—	sprayed with acetate buffer*	I. water II. acetate buffer* saturated with 1-butanol	1-butanol saturated with acetate buffer* pH 5.6	1% PABA in ethanol, then CNBr vapour
E) Kraft (1953)	WF_1 produced by Gessner and Kreuzig, Niederschlag, Erzgeb. Ger.	—	I. 0.1N glycocoll-HCl, best sep. pH 8.7 II. 0.2 M borate buffer**, best sep. pH 7.8 III. phosphate buffer, best. sep. pH 7.8 IV. citrate-HCl buffer, best. sep. pH 6.5	water saturated n-butanol	water saturated n-butanol	Dragendorff, if chromatograms are basic are placed into acetic acid vapour before treated with the reagent
F) Papp—Szabó (1963)	Silicagel G nach Stahl	—	0.5 N KOH	—	toluene-methanol-chloroform 90 : 30 : 10	Dragendorff, modified by Munier, and made more sensitive by using the method of Vágujfalvi (1960)
G) Speak—McCloskey—Smith—Scott—Hussey (1964)	Kieselgel G (Merck)	—	—	—	methanol	Dragendorff reagent
H) Fejér-Kossey (1964)	Silicagel (E. Merck)	—	0.5 N KOH	chloroform ethanol 90 : 10	chloroform ethanol 90 : 10	Dragendorff reagent; 2% aniline in ethanol, or 1% benzidine in methanol, then CNBr vapour different colouration

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* Acetate buffer according to Porter, Naghski and Eisner (1949); 0.2 M acetic acid 95 ml, 0.2 M sodium acetate 90.5 ml.

** 0.2 M borate buffer according to Sörensen and Clark

Table 1/B (cont.)

Author	Paper or thin-layer	Preparation of				Detection
		extract or authentic substances	stationary phase	vapour atmosphere used for	mobile phase (solvent system)	
		with				
I) WINEFORDNER—MOYE (1964)	aluminium-oxide type G (Brinkman)	—	—	—	chloroform-methanol 100 : 1.5	Dragendorff reagent
J) LEETE (1968)	Silicagel F_{254} (Merck)	—	—	—	chloroform-methanol 40 : 10	—
K) LOVKOVA—MINOZHEDINOVA (1969)						Dragendorff reagent
L) HODGSON—SMITH—GUTHRIE (1965)	Silicagel G				I. chloroform-methanol-ammonia 60 : 10 : 1 II. chloroform-methanol-acetic acid 60 : 10 : 1	2% PABA in ethanol and 0.1 M phosphate buffer pH 7.0 1 : 1 then CNBr vapour
M) FEJÉR-KOSSEY (1967)	Silicagel G (Merck)		0.5 N KOH		I. chloroform-methanol 100 : 20 II. chloroform-ether-tetrahydrofuran 80 : 15 : 5	Dragendorff reagent or 1% benzidine in ethanol then CNBr vapour

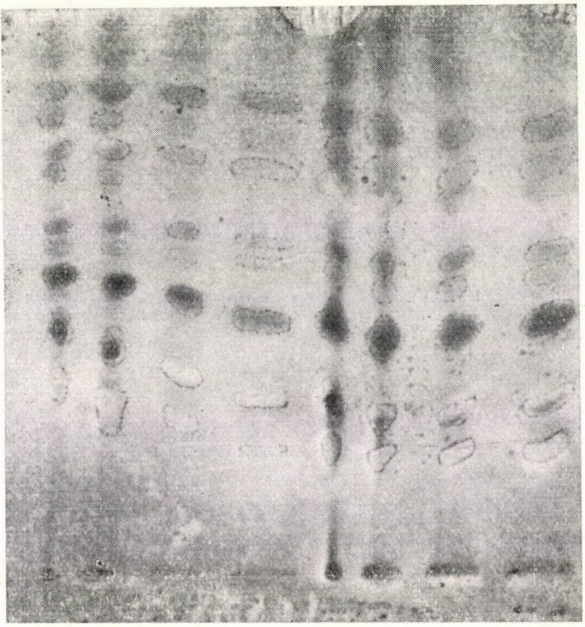

Fig. 1b

Fig. 1. The separation of alkaloids when the extract is placed on the layer in spots and lines. From point 1 to 4, extracts corresponding to 0.25 g air dry weight of tobacco leaf powder, from 5—8, extracts corresponding to 0.5 g, were placed on the Silicagel G (Merck) thin-layer. (20 × 20 cm plate, cubic capacity of the tank 4200 ccm, solvent system 2, front level 6, 10, 16 cm, vapour development 30 min., equilibration of the layer 30 min., detection Dragendorff r. modified by Munier.) *a)* a drawing and *b)* a photography of the TL-chromatogram

responding to the spots or stripes already identified can be scraped off, eluted and subjected to quantitative determination. By using the two dimensional method, even if some spots migrated at a greater distance from each other, the application of the above principle (for non-fluorescent alkaloids) would be impossible.

I have already used this principle successfully in separating opium alkaloids by paper chromatography and later on by thin-layer chromatography (FARKAS-RIEDEL, unpublished results). I applied the samples in a start line (instead of spots) and used three different solvent systems in consecutive runs, as also described later by HALPAAP (1963). I found that the application of the samples on a 3 cm long start line gives satisfactory results if 5 ×20 cm plates are used, and the amount of extract is 0.5 ml (corresponding to 0.5 g of ground poppy capsules). After the runs, the chromatograms were treated with reagents on one side in a width of 1/2 —1 cm. The other (non-reacted) edges of the chromatograms were used for the quantitative photometric determination of the alkaloids after their elution from the corresponding area of the chromatogram. These observations were applied for the tobacco alkaloids and the results are described in the present paper. In this case the chromatograms were run twice or three times consecutively in different solvent systems by using the same start point or line and by running the chromatograms to different distances.

This method is similar to some extent to the polyzonal thin-layer chromatographic method of NIEDERWIESER—BRENNER (1965a) in which the chromatograms are run in multicomponent solvent systems from different start points to the same front distance.

Material and Method

Two solvent systems are described in the present paper. In order to obtain consistently good results with both of them, the conditions prescribed for the run are very strictly adhered to. The R_f-value, even in the case of a one solvent system and a single run, is greatly affected by a number of factors, such as the composition of the solvent systems, the constant temperature, the size of the tank, the extent of vapour saturation, the degree of the activity of the layer, the equilibration of the layer and the chamber.

In Fig. 2 the separation of alkaloids as obtained with only one solvent system are shown as a function of the front level.

Table 2 presents the R_f-values (average of ten replicates) obtained under identical circumstances by one solvent system and different front distances.

In spite of the influencing factors, mentioned above, being entirely identical, the R_f-value is not suitable for the identification of substances in multicomponent systems after several consecutive runs. Under these circumstances the R_f-values only indicate a relative

Table 2

R_f-values × 100 of the separated tobacco alkaloids from cured tobacco leaf extract. One solvent system (chloroform-ethanol-formamide, 90.: 10 : 4 by vol.) in one and two runs and different front distances. (For details see Fig. 2)

Alkaloids separated (numbering of spots from below)*	R_f-values × 100, if distance from start to front is		
	10 cm	16 cm	10 cm and consecutively 16 cm
10	83	70	89
9	72	63	84
8	66	54	77
7	51	47	70
6		36	60
5	36	31	55
4	30	25	46
3	24	19	36
2	17	12	29
1	2.5	2	5

* Numbering refers to the separation as shown in Fig. 2 columns I, II and III.

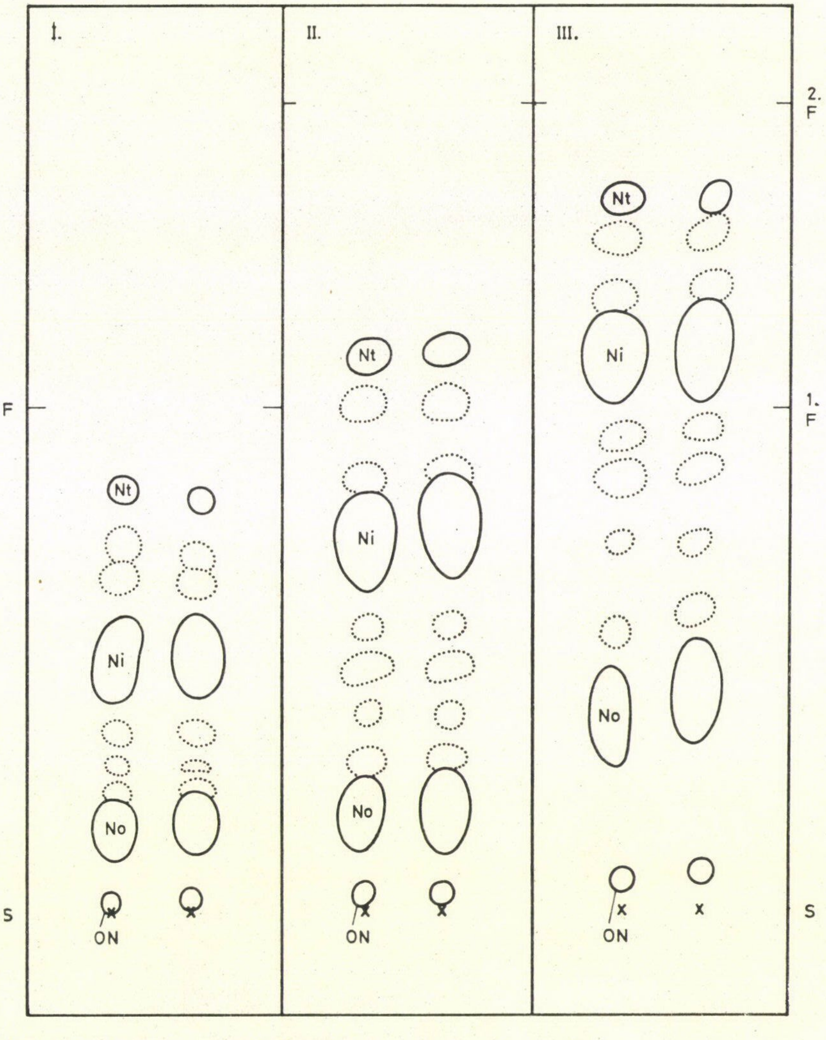

Fig. 2. Location of the separated alkaloid spots from cured tobacco extract by using only one solvent system (chloroform-ethanol-formamide, 90 : 10 : 4) with various distances in one and two runs. (5 × 20 cm plates, cubic capacity of the tank 6200 ccm, vapour develop 30 min., equilibration 30 min., detection Dragendorff r.)

distance of the alkaloid spots from each other, i.e. the sequence of spots on the chromatogram, which has to be verified by using authentic samples and colour reagents.

The same solvent system (chloroform-ethanol-formamide, 90 : 10 : 4) was used for running two-dimensional chromatograms as well, in one direction to a distance of 10 cm and in the second direction to a distance of 16 cm. This procedure did not improve the extent of separation significantly. Therefore, the one-dimensional method, more suitable for routine work, was improved in further experiments.

Thin-layer chromatography was done with the method described by STAHL (1962, 1967) and used also by FEJÉR-KOSSEY (1964, 1967) and other authors, on Silicagel G (Merck) coated plates. The slurry was prepared with distilled water instead of 0.5 N potassium hydroxide. The chromatographic tank was embedded with filter paper to ensure the necessary vapour tension. The solvent system was poured after thorough shaking into a tank of 6200 cm^3 cubic capacity, which was kept for 30 min. at 20°C to obtain the full saturation and for an additional 30 min. for equilibration. After every run the plates were dried at least half an hour by aeration. The neutral extracts from cured tobacco leaves containing a relatively small amount of nicotine, received from the Tobacco Factory, Debrecen, were used only for qualitative assay separated at 8.5 pH by chloroform in 2 spots each on 5 × 20 cm plate, in ten replicates.

The multicomponent solvent systems for qualitative and semi-quantitative determination are;

1. *Chloroform-ethanol-25%* NH_4OH*, 90 : 10 : 2 by vol.* in the first run till a front distance of 12 cm, followed by a second run in *chloroform-ethanol-glacial acetic acid, 90 : 10 : 2 by vol.* till a front distance of 12 cm.

2. *Chloroform-ethanol-formamide, 90 : 10 : 4 by vol.* in the first run till a front distance of 6 cm, followed by a second run in *chloroform-ethanol-25%* NH_4OH*, 90 : 10 : 2 by vol.* till a front distance of 10 cm, and followed by the third run in *chloroform-ethanol-glacial acetic acid, 90 : 10 : 2 by vol.* till a front distance of 14 cm (Fig. 3).

After drying the chromatograms for at least half an hour, the visualization of the spots takes place by spraying the plates with the most sensitive Dragendorff reagent modified by Munier. The detection of the alkaloids can also take place by the application of 1% benzidine in ethanol followed by treatment with BrCN vapour (FEJÉR-KOSSEY 1967) or by spraying with 2% alcoholic solution of p-aminobenzoic acid (PABA) and 0.1 M phosphate buffer, pH 7.0 mixed 1 : 1, followed also by the development of the plates in BrCN vapour as described by HODGSON—SMITH—GUTHRIE (1965). In these cases, in contrast to the treatment with Dragendorff reagent, the spots representing various alkaloids appear not only in different intensities but also in different colours, but within a very short time they turn pale. For the detection of nornicotine the specific isatin reagents of MICHL—KUHN—BÜHN (1956), KUHN (1958) and STEPHENS—WEYBREW (1959) were used. Most of the spots were identified on the basis of

Table 3

R_f-values × 100 of the tobacco alkaloids from cured tobacco leaf extract separated by solvent system 1 and 2

Alkaloids (numbering of spots from below)*	R_f-value × 100 in solvent system			
	1	identified alkaloids	2	identified alkaloids
13	—	—	86	
12	—	—	82	
11	86		77	Nt
10	75		71	
9	68	Nt	67	
8	61	+	64	
7	53	+	61	
6	45	Ni^{+}	56	
5	33	At	49	Ni
4	25	Ab	36	At
3	19		24	Ab
2	13	No	10	No^{+}
1	0.5	ON	2	ON

* Numbering refers to the separation as shown in Fig. 3.

Abbreviation of alkaloids in this paper; N-oxynicotine = ON; nornicotine = No; anabasine = Ab; anatabine = At; nicotine = Ni; 2,3'-dipyridyl = Dp; nicotyrine = Nt; $^{+}$ = means, the spot contains other alkaloids not separated.

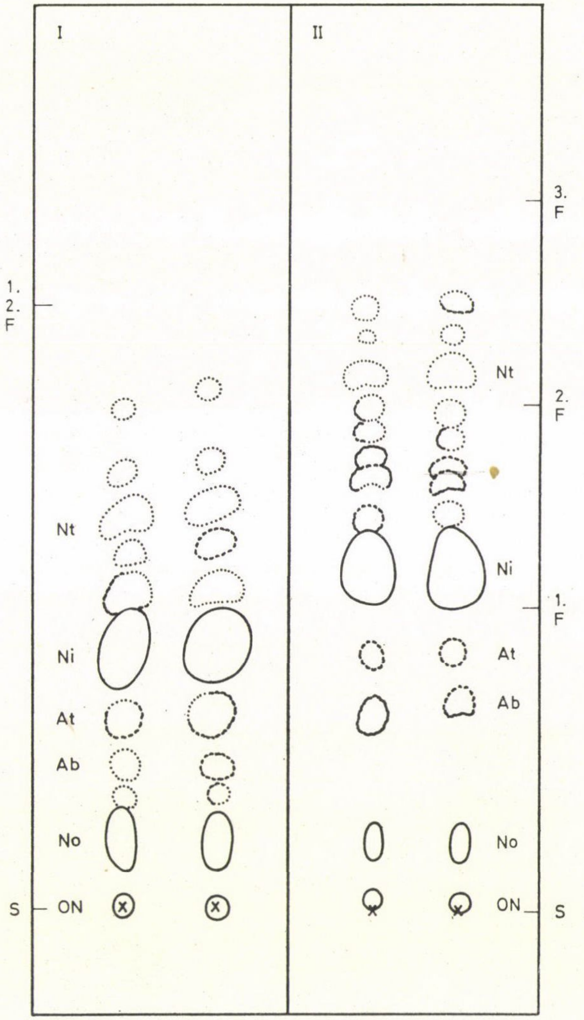

Fig. 3. Chromatograms obtained by solvent system 1 and 2 from cured tobacco leaf extract. (The same circumstances as in Fig. 2)

literary data. Since authentic substances were not at my disposal, the identification was limited to N-oxynicotine, nornicotine and nicotine. The two spots, nicotine and oxynicotine, could be eluted separately and one was oxidized the other reduced according to FRANKENBURG—GOTTSCHO (1955). The R_f-values obtained by the two new solvent systems are presented in Table 3.

Results

The results presented in this paper are summarized in Table 4.

Studies which have led to the development of the two multicomponent solvent systems described in the present paper and investigations on the quantitative assay of the separated alkaloids had been made necessary by experiments on the process of demethylation in tobacco leaves. The two solvent systems, applicable to thin-layer chromatography, make a rapid separation of

Table 4

Number of spots separated by TLC in different solvent systems (1 and 2) in consecutive runs and the quality of separation

Solvent systems	Number of spots separated	Separation of spots except nicotine and nornicotine			Front distance in consecutive runs in cm
		below nornicotine	between nornicotine and nicotine	above nicotine	
1	11	1 good	3 very good	5 very good	12, 12
2	13	1 good	2 good	8 excellent	6, 10, 14

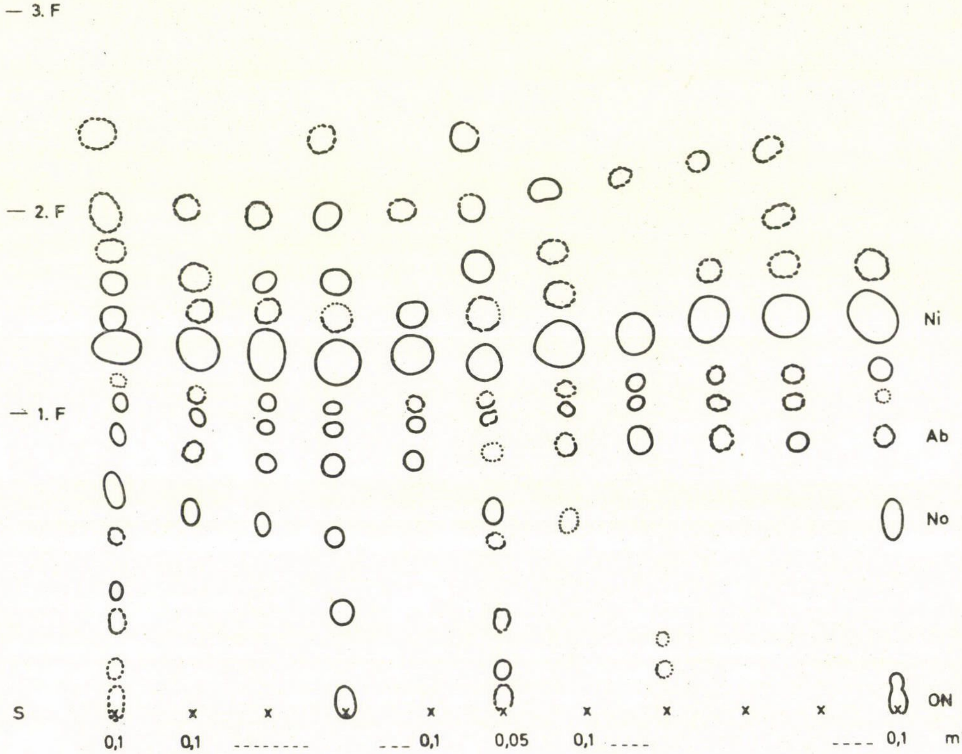

Fig. 4a

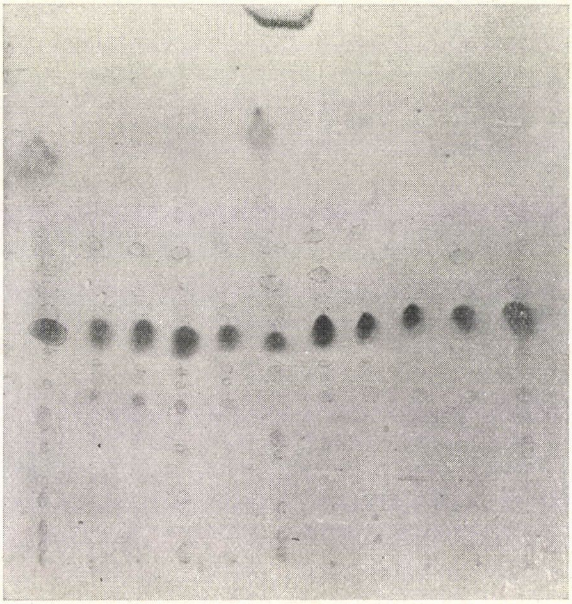

Fig. 4b

Fig. 4. The application of solvent system 2 used for routine work within the framework of extraction experiments. Every starting point received 0.1 ml extract corresponding to 0.1 g air dry weight of cured tobacco leaf powder. (The chromatographic circumstances correspond to those of Fig. 1, but in this case the front levels are suitable for the description 6, 10 and 14 cm. *a)* a drawing of the chromatogram, *b)* a photography of the same chromatogram

10—13 or more alkaloid components possible, but only for qualitative and semi-quantitative determination. Fig. 4 shows the separation capacity of the solvent system No 2. The emphasis was laid on the good separation of nornicotine and nicotine from their neighbours. This is shown in Table 4. By emphasizing the number and distance of neighbouring spots, I draw the attention to the fact that the higher the number of separated spots and the greater their distance from nicotine and nornicotine the more likely it is that the eluates of the spots corresponding to nicotine and nornicotine will not contain other alkaloids. In this way the quantitative photometric data are certainly more reliable. The description of newer solvent systems which are able to separate the alkaloid components from each other to a higher extent will follow in a next paper.

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CHROMATOGRAPHIC HETEROGENEITY OF RED AND WHITE RABBIT SKELETAL MUSCLE MYOSIN

By

S. Fazekas, V. Székessy-Hermann, I. Kása, M. Szamel, K. Szabó

INSTITUTE OF MEDICAL BIOCHEMISTRY, SEMMELWEIS UNIVERSITY OF MEDICINE;
INSTITUTE FOR APPLIED CHEMISTRY OF THE TECHNICAL UNIVERSITY, BUDAPEST

The paper deals with the heterogeneity of red and white rabbit muscle myosins. The individual red and white muscles have been found to contain different and variable myosin subfractions. In general, the red skeletal muscle myosins are characterized by the presence of fractions I and II, while the white ones by fractions III, IV, V and VI. There are, however, differences in the myosin fractions between the individual muscles, since any of the four fractions of the white muscle may be absent, and even their ratio is highly varied. All subfractions have a characteristic Ca^{2+} induced ATP-ase activity. ATP-ase activity and fluorescence intensity in the subfractions of red muscles are inferior to those in the subfractions of white muscles, and the secondary matter seen on its chromatograms is more than in the myosins of white muscles. The lipid content is 20—40 per cent higher in the red than in the white muscles.

Introduction

Bárány (1967) was the first to point out that the ATP-ase activity of myosins originating from different organisms was varied but by and large proportional to the contraction time of the respective muscle; that is, the quicker the contraction, the higher the ATP-ase activity. Recently it has been mentioned more and more frequently that within the same animal species the ATP-ase activity found in the myosins also varies according to the white (fast) and red (slow) type of muscle (Bárány *et al.* 1965, Sprony 1968, Kim—Mommaerts 1971). Trayer—Perry (1966) found myosin fractions possessing different ATP-ase activities in the muscles of new-born and adult rabbits. Although the sedimentation constants were identical, still, on the basis of their experiments, they considered the myosin to be a mixture of heterogeneous molecules. In our previous paper (Fazekas *et al.* 1971) we pointed out that from the mixed skeletal muscles of rabbit at least four chromatographic fractions showing ATP-ase activity could be separated on a DEAE-cellulose column.

According to Kim—Mommaerts (1971) the various type myosins differ from one another in the number of light components namely, minor components isolated with 4 M KCl from slow muscles showed three, while those from fast muscles four electrophoretically separable components. The same results were obtained by Perrie—Perry (1970), but in the cardiac muscle myosin they found only two light components. The low molecular weight components of myosins obtained from the skeletal and cardiac muscles of several animal species were compared earlier by Locker—Hagyard (1967) who found two and three light components, respectively, in the different origin muscles. From the location of the low molecular weight components one can conclude on their not having the same characteristics.

Therefore the question arises whether the myosins obtained from individual muscles of species show a single chromatographic myosin fraction, or various myosins occur from the beginning in the individual muscles, or again, the myosins differ according to the fast and slow muscles only. A further question is whether chromatographic fractions obtained from the individual muscles contain low molecular weight components. To decide on the questions arisen we obtained myosin from a single muscle, while in another case we prepared a so-called mixed myosin from mere red and mere white muscles, respectively, and separated their chromatographic fractions in order to be able to compare their chromatograms and some characteristics of the fractions.

Material and Method

For the purpose of experiments muscles of rabbits not older than four months were used. The back half of the body of exsanguine rabbits was removed as soon as possible and cooled in ice. The required muscles of the hind legs were quickly excised and classified on the basis of their visible colour as white, red or pink. The muscles were finely ground with a La Tapie-type grinder, weighed, and the myosin was isolated according to Portzehl—Schramm—Weber (1950) as modified by Szent-Györgyi (1951). According to the method the myosin was precipitated with a twelvefold dilution, while actomyosin was removed at a concentration of 0.26 M KCl. Purification was repeated once by cyclic precipitation. Prior to the chromatographic separation myosin was purified in an ultracentrifuge (105 000 × g,1h,)* and the transparent supernatant was prepared for chromatographic separation by dialysing the myosin containing 1 mM 2-mercaptoethanol against 0.02 M pyrophosphate buffer (pH 7.4). The buffer was changed four times; the optical density of myosin measured and separated — as described earlier (Fazekas *et al.* 1971) — on DEAE-cellulose (Whatman DE 32) column activated and balanced with 0.01 M pyrophosphate buffer. The eluents are shown in the figures. Fluorescence studies were performed by means of a HITACHI MPF-2A spectrofluorometer.

Results

In part of the experiments only red muscles (*m. soleus*, *gastrocnemius*, *ischiotibialis*, *semitendinosus*) were used, and the myosin obtained from them was separated on DEAE-cellulose column. The result is shown in Fig. 1.

The eluent solutions are indicated on the chromatogram, but the activity of the peak tubes of the individual fractions are seen in Table 3 expressed in μ moles Pi/mg protein/minute. The table shows that in the red muscles there are five myosin fractions showing ATP-ase activity. Table 1 summarizes the recov-

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* spinco ultracentrifuge-model L.

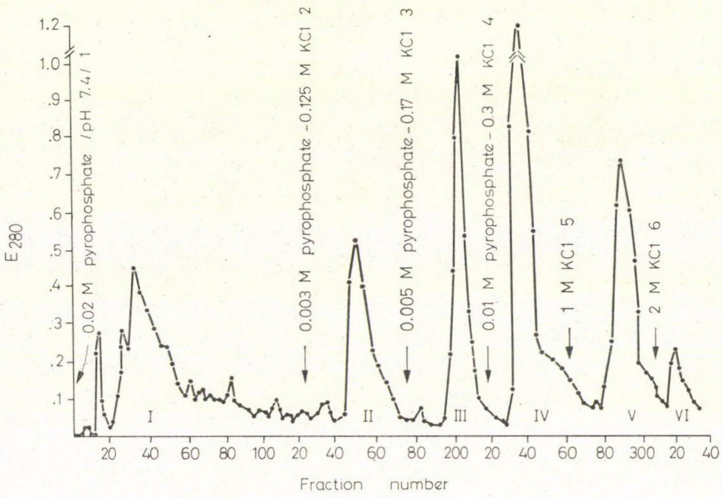

Fig. 1. Chromatography of mixed red muscle myosins on DEAE-cellulose column

Table 1

Recovery of red myosins from the chromatographic column and their percentage distribution

Number of fractions	E_{280}	%
12— 17	3.8	1.52
18— 20	0.54	0.5
21— 31	12.28	4.9
32— 61 I.	48.47	19.3
62—143	31.99	12.75
144—187 II.	33.34	13.4
188—228 III.	40.5	16.6
229—260 IV.	58.34	23.3
261—280 V.	33.57	13.5
281—294 VI.	6.8	2.71
recovery	269.3	107.—
On the column	251.2	100.—

ery of the myosins from the chromatographic column and their percentage distribution.

The myosins produced from the individual muscles were subsequently applied to DEAE-cellulose columns of possibly identical volume. The chromatograms thus obtained are suitable for comparison. Fig. 2 shows the chromatograms of four red muscles separately, indicating the respective eluating solutions.

When comparing the chromatograms we can see that the fractions occur in different quantities in the individual muscles. Fraction I is found in the largest quantities in the red m. ischiotibialis and soleus, while in the pink semitendinosus and gastrocnemius its quantity is small. All four muscles are characterized by the presence of the relatively high activity fraction V, while fraction IV is significant only in the gastrocnemius.

The chromatogram of myosin obtained from mixed white femoral muscles is seen in Fig. 3. The mixture contains the muscles of *M. gluteus medius, semimembranosus, quadriceps, biceps femoris* and *vastus.*

On the chromatogram, fraction I is completely missing, only slight traces of contamination are seen in its place. Fraction II amounts only to 9.1 per cent of the material applied. Fraction VI not characteristic of the red muscles is also found in the white muscles, though a considerable part of this fraction

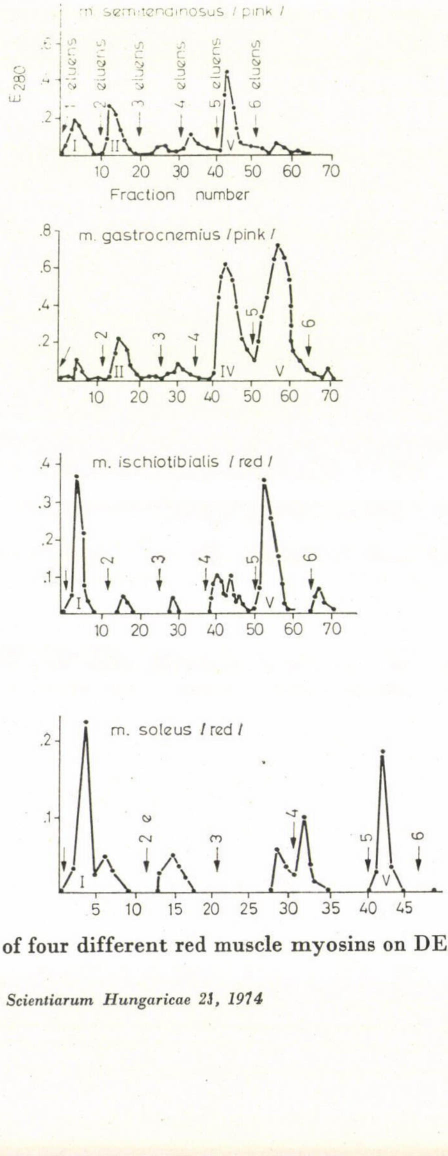

Fig. 2. Chromatography of four different red muscle myosins on DEAEA-cellulose column

Table 2

Distribution and percentage recovery of chromatographic fractions of mixed white muscle myosins

Number of fractions	E_{280}	%
0— 20	1.0	0.5
20— 50	4.9	2.6
51— 68	1.6	1.0
69— 90 II	17.6	9.1
91—111 III	28.3	14.7
112—133 IV	35.2	18.1
134—155 V	38.3	20.0
156—185 VI	43.1	22.4
with alkali	21.0	10.8
recovery	191	99.6
on column	192 E_{280}	100.—

Table 3

μ mole Pi/mg protein/minute value of specific ATP-ase activity observed in the peak tubes of chromatographic fractions

Muscle	O fr.	I.	II.	III.	IV.	V.
red (mixed)	0.125	0,06	0.077	0.17	0.065	0.115
white (mixed)	0.28		0.11	0.35	0.39	0.180
m. biceps femoris (transitional)	0.125	0,185	0.445 0.575	0.37	0.275	0.185

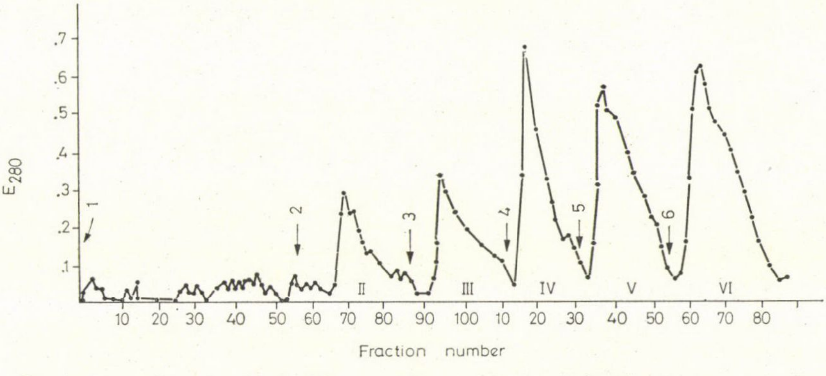

Fig. 3. **Chromatography of mixed white muscle myosins on DEAE-cellulose column**

is lipid. Table 2 shows the distribution of myosin fractions and their recovery percentage from the chromatographic column.

Fig. 4 shows chromatograms of myosins obtained from muscles perfectly white to the eye. Fractions I and II are completely missing from the chromatograms of each muscle; fraction III occurs only in myosin produced from the semimembranosus and even there in two separate peaks.

Fig. 5 shows the chromatogram of myosin obtained from *m. biceps femoris*. At the beginning of the procedure the myosin of this muscle was treated as a white muscle myosin, but on the basis of its chromatogram it could not be placed among the myosins of Fig. 4. Namely the chromatogram shows a transitional type, that is the reason why it is illustrated separately. In the figure fractions I, II and III together amount to some 25 per cent, and the odd thing about it is that fraction II eluates in two separate peaks and exhibits different specific activities.

Fractions of myosins isolated from red and white muscles have different ATP-ase activities. Table 3 summarizes the specific ATP-ase activities observed in μg Pi/mg protein/minute in the peak tubes of chromatographic fractions.

The Ca induced ATP-ase activity of myosin was determined by the method of HOLLAND—PERRY (1969) by measuring the quantity of anorganic phosphate originating from the splitting of the ATP.

The myosin fractions of the different muscles were placed in 8 M urea and gel filtrated on Sephadex G 200 column also equilibrated in urea, with the aim of separating the large polypeptide chain- and low molecular weight components of the myosin. The gel-filtration chromatograms disclose that all myosin fractions obtained on DEAE-cellulose column contain the heavy and light components though in highly different proportions. Fraction I of the red muscles contain a relatively low amount of heavy and large amount of small subunits of myosin. The area of peaks drawn on the basis of the E_{280} absorption of the high and low molecular weight components of the chromatographic fractions II and III is larger for the light components, while in fraction IV it is the area of the heavy component that dominates more strongly. In fraction V of the red and white muscle myosins the ratio of the two components is highly different; e.g. in the gastrocnemius (red) muscle the area of the light components, while in the white muscle that of the heavy component is larger. Fig. 6 summarizes what have been said. The gel-filtration chromatograms of fractions IV and of the white fraction V are similar. The forms of the small components are varying which suggests that they consist of a number of components according to their molecular weights, but those of the red muscles are always more homogeneous.

We examined and compared fluorescence in single and mixed muscle myosins. Both excitation and fluorescence values were related to the same E_{280} value, so the various intensities could be compared within the same figure.

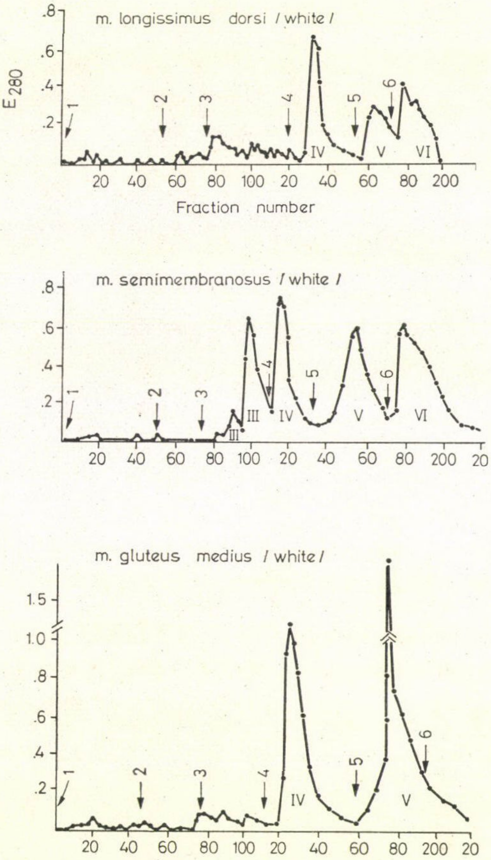

Fig. 4. **Chromatography of three different white muscle myosins on DEAE-cellulose column**

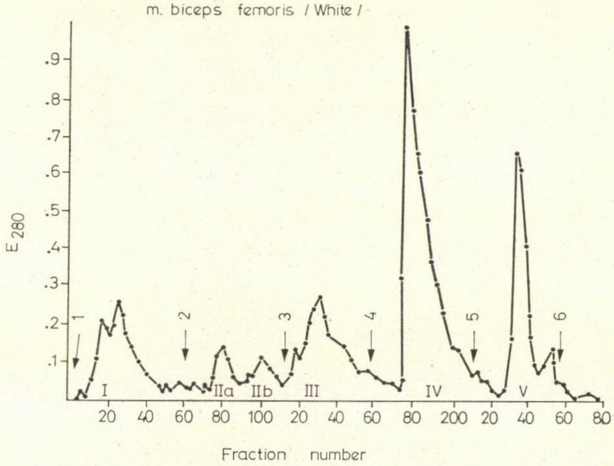

Fig. 5. **Chromatography of m. biceps femoris "white" myosin on DEAE-cellulose column**

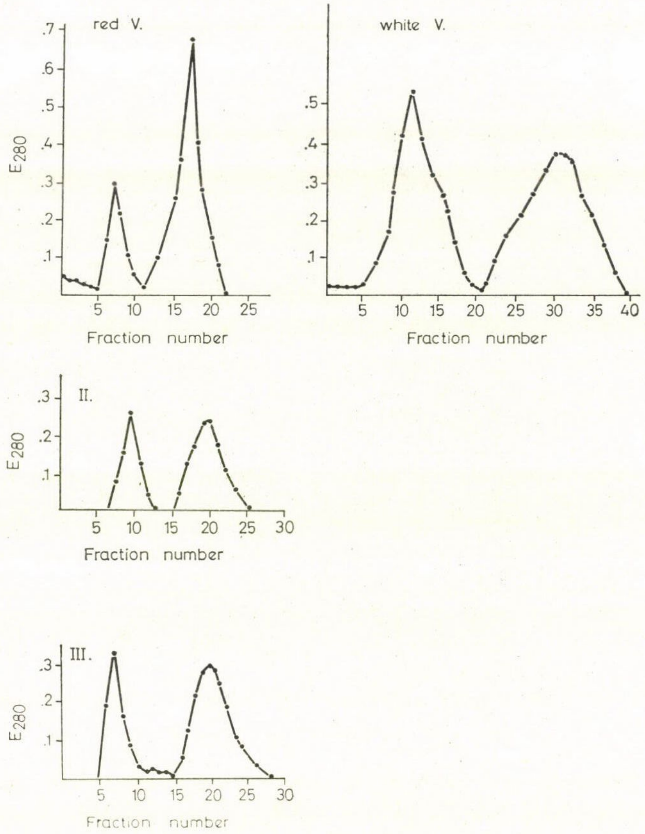

Fig. 6. Gel-filtration of mixed red muscle myosins on Sephadex G-200 column, equilibrated in 6 M urea

Fig. 7 shows the excitation and fluorescence spectra of mixed red (R_0) and transitional m. biceps femoris (F_0) myosins, before chromatographic separation. We can see that the intensity of biceps myosin is lower.

Fig. 8 shows the excitation and fluorescence spectra of chromatographic fractions of mixed red myosin according to the peak tubes of Fig. 1. Tube 13 is 2-mercaptoethanol and it shows no fluorescence. Tubes 28 and 35 fall within the area of fraction I. As it was reported earlier (FAZEKAS *et al.* 1971) it is a mixture of several proteins and secondary matters. It is mainly AMP-deaminase and acetyl-choline-esterase enzymes accompanying the myosin that are found here. Tubes 150 (II), 191 (III), 231 (IV) show the intensely fluorescing myosin fractions, while tubes 265 and 282 the lower intensity and higher lipid content fraction V which, however, possess considerable ATP-ase activities.

Fig. 9 compares the excitation and fluorescence spectra of myosins from two red muscles, those of *soleus* (S_0) and *ischiotibialis* (I_0), before the chroma-

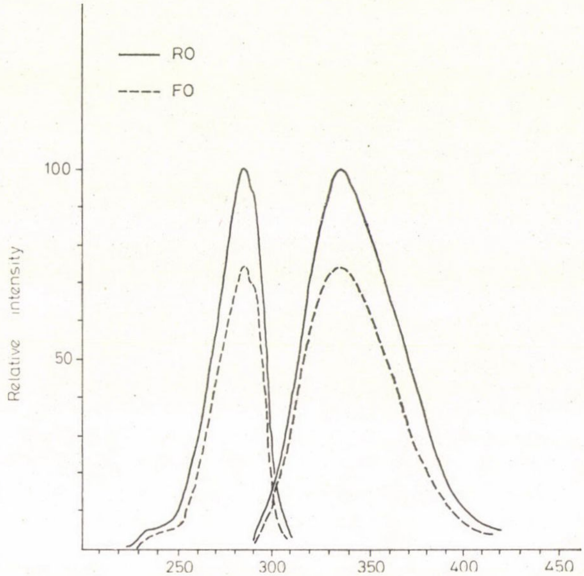

Fig. 7. Excitation and fluorescence spectra of red (Ro) and "white" biceps femoris (Fo) myosins before the chromatographical procedure

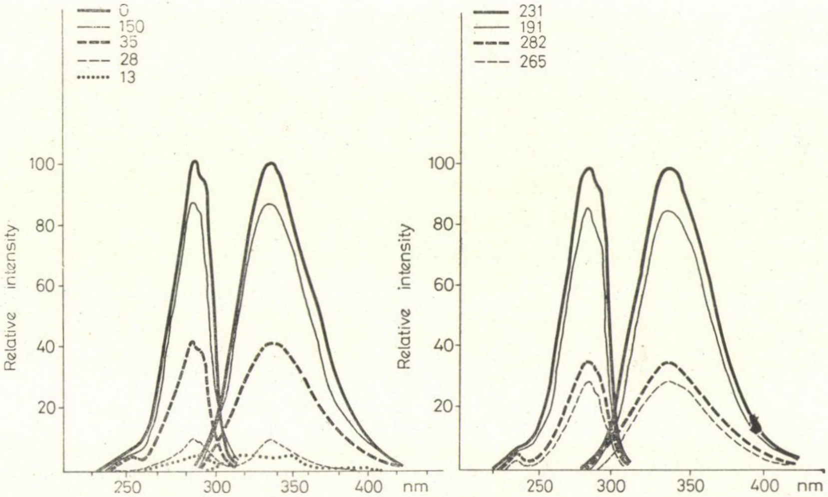

Fig. 8. Excitation and fluorescence spectra of mixed red myosin fractions by peak tubes

tographic separation. When related to the same E_{280} value the intensity of *ischiotibialis* myosin is found to be about twice as high as that of the myosin of *soleus*, and in its fluorescence spectrum a peak around 305 nm can be seen clearly.

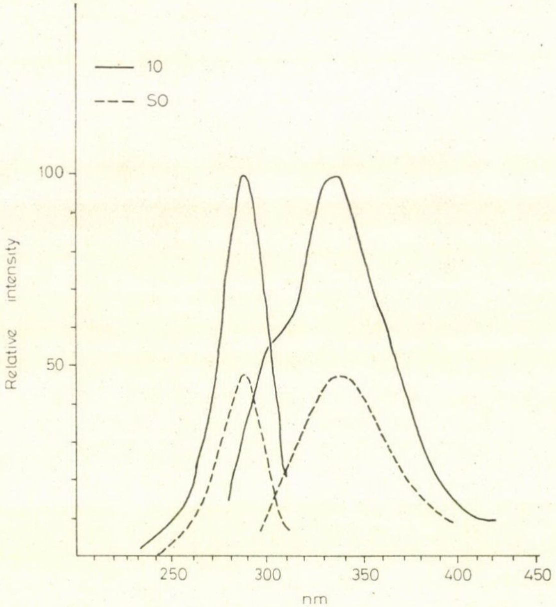

Fig. 9. Excitation and fluorescence spectra of m. soleus and ischiotibialis myosins before the chromatographic separation

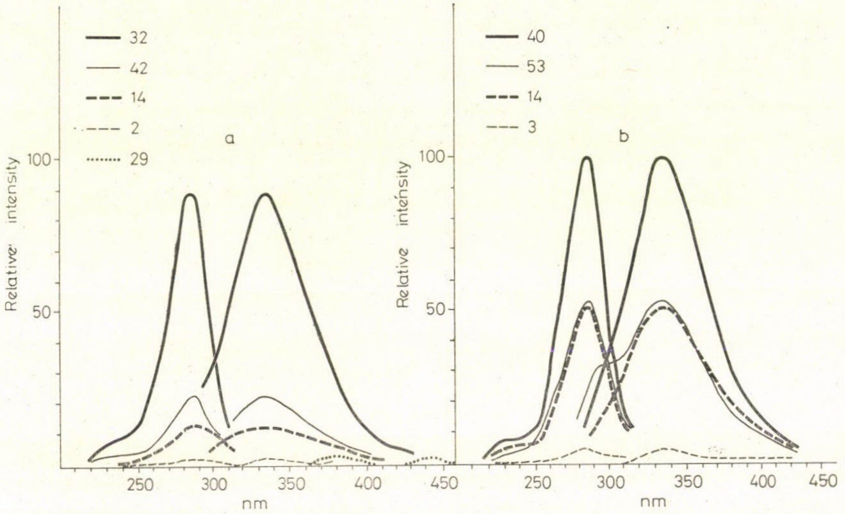

Fig. 10. Excitation and fluorescence spectra of soleus (a) and ischiotibialis (b) myosin fractions by peak tubes

Fig. 10a shows the excitation and fluorescence spectra of *soleus* myosin fractions. Only tube 32 (IV) shows intensive fluorescence, the others, including tube 42 (V), hardly fluoresce. Tube 29 has no myosin; in a separate investigation it was found to contain a lipid.

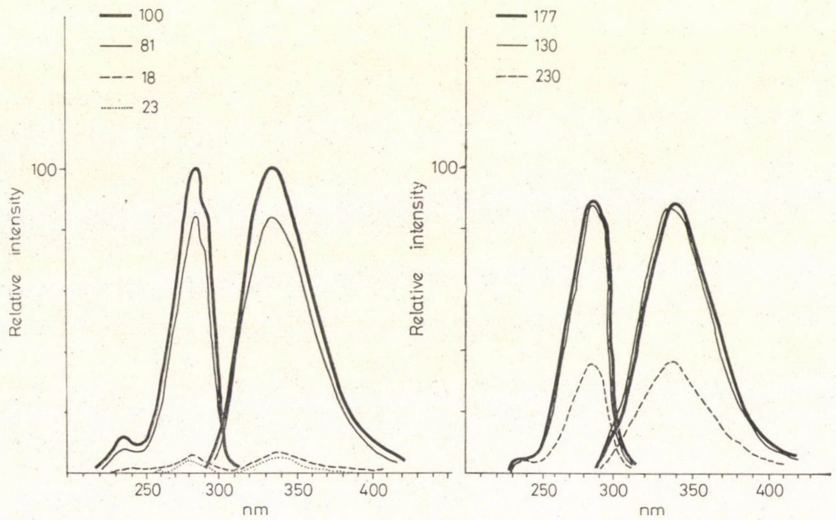

Fig. 11. Excitation and fluorescence spectra of biceps femoris myosin fractions by peak tubes

Fig. 10b shows the spectra of *ischiotibialis* myosin fractions. The most intensive fluorescence is found with tube 40 (IV) while tube 53 (V) hardly shows one third of the former's intensity. The figure indicates that the shoulder observed in Fig. 9 around 305 nm after the separation belongs exclusively to fraction V.

Fig. 11 shows the spectra of *biceps femoris* myosin. Tubes 81 (IIa), 100 (IIb), 130 (III), and 177 (IV) fluoresce intensely, tube 230 (V) only with about one-third intensity. The fluorescence intensity of fraction I is very low. The fluorescence intensity of the myosins of white muscles corresponds to that of fractions II, III, IV and V seen in Fig. 11 with the exception that in fraction VI it is still lower than in fraction V. Its high tendency to peroxidation suggests that it consists mainly of lipids.

Discussion

In our investigations we tried to find an answer to the question whether there is only a single myosin or various kinds of myosin occur in the muscle or in a mixture of muscles. Our results show that there are various kinds of myosin in the skeletal muscles, or at least, myosins obtained from both red and white muscles can be separated into a number of chromatographic fractions.

The ATP-ase activity in the chromatographic fractions of red muscle myosins is always inferior to that in the myosin fractions of white muscles. The myosins of red muscles are characterized by the presence of fractions I and II which do not occur in the typical white muscles. These are the fractions which beside a low ATP-ase activity show AMP-deaminase- and acetylcholine-esterase activities accompanying the myosin in the mixed muscles (FAZEKAS *et al.* 1972). Fraction VI which is characteristic of the myosin of white muscles is not contained in the red muscles. There are so great differences in occurrence and percentage distribution between the chromatographic fractions of individual red and white muscles that the existence of independent myosin fractions in the individual muscles may be taken in consideration. Fraction I and usually fraction II too are missing in the myosins of white muscles, and fractions III, IV, V and VI are not always present in all muscles either. Beyond the chromatographic fractions of the white muscles there also exist lipids (fatty acids) eluable with 2—15 per cent alkaline solutions but not with 2 M KCl. We mention here that beside the white muscles containing 2—4 fractions shown in the figures there are white muscles which contain but a single main chromatographic fraction, as e.g. *m. longissimus* and *m. psoas*. It is thus only the chromatographic fractions of homologous muscles that can be compared in different animal species.

Fractions of both red and white muscle myosins can be separated into two components — heavy and light fractions — by the method of gel filtration, in 8 M urea. The ratio of high and low molecular weight fractions varies considerably in the different muscles. Fractions I and II of the red muscle myosin show less heavy and more light components, while when examining fraction V in red and white muscles we find that in fraction V of the red muscle there occur light, and in that of the white muscle heavy fractions in larger quantities.

It is worth mentioning that the amount of isolable lipids is different in the two types of muscle. While from mixed red muscles 2.0—2.2 g, from white muscles only 1.6—2.0 g lipid/100 g fresh muscle can be isolated. In the red muscles 16—19 mg, in the white ones only 11—14 mg phospholipid/g muscle are found. As reported in a previous publication (FAZEKAS *et al.*, 1971) chromatographic fractions of the mixed muscle myosin too contain lipids which show characteristic fluorescence spectra different from that of the myosin. As to the existence of some characteristic difference between the lipid contents of the individual myosin fractions no reliable data have been obtained so far, but it is supposed that beside the protein components the lipids too take part in the structural development of the specific myosin molecules of the individual muscles.

We made the excitation and fluorescence spectra of myosin and its separated fractions as fresh as possible in order to avoid the auto-oxidation of the lipids of myosin which occurs around 350 and 440 nm. As shown by the figures of the examined preparations we succeeded in attaining this aim. A comparison of the figures reveals that it is the red muscle that fractions I and II are characteristic of. On the chromatograms of myosins isolated from mixed muscles their area is in connection with the relative proportion of red muscles. Red muscle myosins and fractions show a lower fluorescence and ATP-ase activity than those of the white muscles. On their chromatograms relatively more concomitant substances are found. Their origin — like that of fraction I — is supposed to be in relation with the higher sarcoplasm and mitochondrium contents of the red muscle fibres. In spite of this in the red muscles there are more than one kind of myosin fractions, though with the individual muscles examined only one of them seems to be the main fraction. In the white muscles two or even three fractions may exist simultaneously in nearly the same proportions.

The myosin of biceps femoris was marked as "transitional" between the two kinds of muscle. This is probably justified in the case of the other red muscles too. GAUTHIER (1969) pointed out that the *m. semintendinosus* can obviously be divided into two parts: a red (*anterior*) and a white (*posterior*) part. Both muscular bundles contain mixed fibres the proportions of which change. He found 52 per cent red, 40 per cent transitional and 9 per cent white fibres in the red part, and 4 per cent red, 14 per cent transitional and 82 per cent white fibres in the white part. The red fibres (I. = A) can be easily distinguished by the thick Z-membrane and the mitochondrium between the miofibrils; while the white fibres (II = C) on the basis of the thin Z-membrane and the absence of mitochondrium between the myofibrils. The other muscles of rodents are also supposed to be characterized by the combination of the three fibre types.

According to the complementary gel filtration tests the fraction separated on DEAE-cellulose column consists of intact myosin molecules, and both heavy and light components are present in them, though in various proportions. Thus PERRY's suggestion to mark the myosin fractions with "iso" on the model of the isoenzymes ought perhaps to be accepted.

Our experiments call attention to the fact that only the myosins of single or homologous muscles and their identical subfractions are suitable for analytical, biochemical investigations and comparisons.

Acknowledgement

We are indebted to Mrs. Bajna and Mrs. Bökönyi for their skilful technical assistance.

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INVESTIGATIONS ON THE CORRELATIONS BETWEEN THE RN-ASE ACTIVITY AND ZINC TREATMENT OF AVENA LEAVES

By

J. VETTER

DEPARTMENT OF PLANT PHYSIOLOGY
LORÁND EÖTVÖS UNIVERSITY, BUDAPEST

Changes in the ribonuclease activity of homogenates obtained from leaves of *Avena* seedlings were followed after several hours treatment with zinc and in vitro with zinc present. Incubation in distilled water doubled the total ribonuclease activity of the leaf homogenate; the activity of the variants treated with zinc in the concentration interval of 3×10^{-7}—3×10^{-3}M was lower than this. It was only in the interval of 3×10^{-5}—3×10^{-3} M that the zinc present in the reaction mixture inhibited to a considerable extent the RNA decomposing ability of the homogenates derived from the leaves of the control (untreated) plant. The zinc, as one of the microelements of plants, besides its manifold role (auxin biosynthesis, morphological symptoms, component of enzymes) seems to have a direct effect on the enzymatic factors of growth regulation.

Introduction

On the basis of recent investigations the nucleic acid decomposing enzymes seem to have a role in growth regulation. (LONTAI 1971, MARÓTI 1969, PILET – BRAUN 1970). With hormonal treatments carried out under various conditions the nuclease activity can be both stimulated and inhibited (DOVE 1971, SODEK—WRIGHT 1969, VETTER 1972a, WYEN *et al.* 1969). Some of the nucleases have at the same time become subject of more detailed biochemical study and characterization (UDVARDY—FARKAS—MARRE 1969, WYEN *et al.* 1969).

Material and Method

Leaves of ten days old oat seedlings raised under normal green-house conditions then incubated in solutions of different zinc content and in distilled water (control), respectively, were used as objects of our experiments. Zinc was applied in the form of zinc-acetate, in a concentration interval (M) of 3×10^{-9}—3×10^{-3}. Material of 750 mg fresh weight per sample taken after five hours of incubation was homogenized — after having been washed in distilled water — in a Potter-Elvehjem-type homogenizator, in an acetate buffer of 0.1 M and pH = 5, with continual cooling. It was sedimented in a Janetzky K-24 type centrifuge for 10 minutes at 8000 rpm, then for 60 minutes at 13,000 rpm. The ribonuclease was determined from the five times diluted supernatant, according to the method described previously (VETTER 1972a). After an adequate dilution the extent of enzyme decomposition was determined by the value of extinction measured at 260 millimicrons.

To determine the effect zinc exercised in vitro on the ribonuclease activity of oat leaves, an extract prepared from the leaves of the control seedlings by the above method (ultimate supernatant) was used. The reaction of the enzyme with the substrate to be decomposed — the RNA — then took place in the presence of the required amount of zinc. Concentrations used here were also 3×10^{-9} — 3×10^{-3} M. Ribonuclease activities were expressed in this case too by the values of extinction measured at 260 millimicrons.

We set it as the objective of our work to study the relation of this growth regulating enzyme system to a mineral nutritive element, the zinc. Our efforts are supported by other investigations of similar character made by us (VETTER 1972b) and by literary data referring to a relationship between zinc and nucleases (ISHI *et al.* 1967; KESSLER—MONSELISE 1959).

Results

In our first series of experiments leaves of ten days old oat plants were incubated in solutions containing zinc at various concentrations, then ribonuclease activity determinations were carried out in the same way. The experimental data are presented in Fig. 1. The activity of homogenates derived from leaves floated on distilled water was measured as a control, and determinations were made from freshly excised leaves too. The activity of a freshly cut plant material was found to be slightly more than 50 per cent of the ribonuclease activity measured after five hours of incubation. Data are given in the percentage of this control. In the case of the first member of the concentration series (3×10^{-9}) the activity was unchanged, while with increasing concentrations of zinc a considerable decrease of activity could be demonstrated. This decrease ranged between 22 and 42 per cent. On an average a 30 per cent lower ribonuclease activity could be found in the concentration interval of 3×10^{-7} — $3\times\times10^{-3}$M compared to the untreated control. It is remarkable that activities measured in the treatments were in no case lower than — in fact did not even reach — the total ribonuclease activity of the homogenate obtained from a freshly excised leaf.

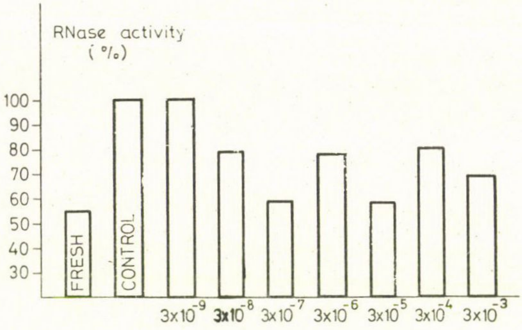

Fig. 1. Effect of zinc treatments on the ribonuclease activity of 10 days old Avena leaves after five hours of incubation [abscisse: zinc concentrations (M); ordinate: ribonuclease activity as a percentage of the distilled water control]

In subsequent experiments the effect zinc exercised in vitro on the ribonuclease activity of oat leaves was studied. In addition to identical amounts of enzyme and substrate the reaction mixture contained zinc too in the con-

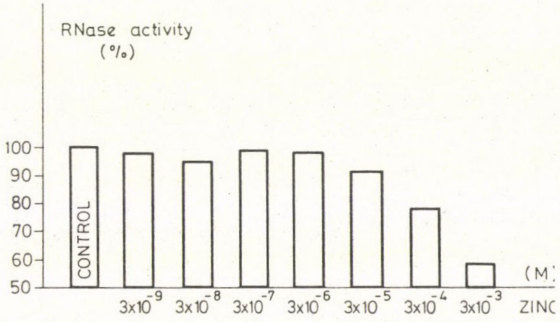

Fig. 2. In vitro effect of zinc on ribonuclease activity in a leaf homogenate from a 10 days old Avena plant [abscisse: variants of zinc concentration (M); ordinate: ribonuclease activity as a percentage of activity measured without zinc]

centration interval of 3×10^{-9} — 3×10^{-3}M, with a variant applied per order of magnitude. The mean values of the activities obtained are shown in Fig. 2. Taking the activity of the zinc-free control to be 100 per cent it can be established that up to a concentration of 3×10^{-6}M the zinc does not essentially influence the RNA decomposing ability. At the same time, a decrease of activity could already be pointed out at 3×10^{-5}, 3×10^{-4} and 3×10^{-3} concentrations.

Discussion

Our experiments were aimed at studying the effect of zinc on the life processes of plants. It is known e.g. that zinc is a stoichiometric component of a number of enzymes; probably plays a role in the early phase of the biosynthesis of the growth regulator auxin (MASEV—KUTACEK 1966), its absence causes clearly visible morphological symptoms (NASON—MCELROY 1963). Earlier experiments performed by us with tobacco tissue cultures revealed that this micro-element exerted either a stimulating or an inhibiting effect — depending on its concentration — on the fresh weight increase, and considerably influenced the trends of respiration intensity and peroxidase activity too (VETTER 1972b). In our present investigations the trend of nuclease activity in the oat leaf was observed at the level of total ribonuclease activity as a first approach. In agreement with other literary data (UDVARDY *et al.* 1969), after five hours of incubation in distilled water the ribonuclease activity of the freshly excised oat leaf increased to a considerable extent. The reason for this is partly the excision itself, partly the distilled water treatment which, as an artificial senescing effect, causes a significant increase in the enzyme activity of nucleic acid decomposition. In earlier investigations LADO *et al.* (1968) pointed out two maxima of ribonuclease activity following the excision of oat leaves; they explained the first maximum with the effect of excision, and the second with the progress of senescence. More intensive examinations isolated a number of nucleic acid decomposing enzymes possessing various characteristics from oat leaves *a)* endo-ribonuclease of relative purin specificity; *b)* non sugar specific endonuclease; *c)* non sugar specific exonuclease (Wyen *et al.* 1972). The latter investigations also pointed to the fact that it is not all the nucleases, but mainly the endoribonuclease decomposing beside the purins that the hormonal effects are quantitatively related with.

Changes occurring under the influence of incubation with zinc (Fig. 1.) can be interpreted by the presence of zinc resulting — in a certain concentration interval — in a lower ribonuclease level after several hours of treatment. It is interesting to compare this fact with data referring to the relation of nucleases and zinc, namely experiments revealing significant differences between healthy and zinc deficient citrus leaves in RNA content, ribonuclease activity and protein nitrogen content were reported as early as in 1959 (Kessler—Monselise 1959). Leaves showing symptoms of zinc deficiency contained less RNA and protein nitrogen, while their ribonuclease activity proved to be higher. Otherwise the zinc was in positive correlation with the RNA and protein synthesis. Taking other results in consideration too the idea arose that as a function of age, there was a close correlation between the different zinc contents (higher in the young and lower in the older plant) and the varying ribonuclease activity; a higher zinc content would have been coupled with low ribonuclease activity and vice versa. In the process of senescence the zinc would have shown a decreasing concentration gradient while the ribonuclease activity an ever increasing tendency.

Our second experiment tried to find an answer to the question of how the measurable activity depended on the quantity of zinc added when the reaction took place (Fig. 2), since the metal ions may be important inhibitors of the activity. It could be demonstrated that between the zinc concentrations of 3×10^{-9} and 3×10^{-5} M inhibition was still insignificant; a strong inhibitory effect could be observed with the 3×10^{-4} and especially the highest concentration 3×10^{-3} M variants (22 and 42 per cent respectively). It is interesting that a very similar effect was found in the case of nuclease in a tobacco callus culture (Vetter 1972c). Results referring to ribonuclease obtained from bean shoots are also remarkable. As it turned out, the $ZnCl_2$ treatment influenced the heat and pH stability of enzymes to a great extent, while other metal ions were ineffective in this respect (Ishi *et al.* 1967). Our results suggest — true, for the time being only on the basis of data obtained at the total ribonuclease level — that the exogenous zinc treatment may have a part in controlling the activity of the nuclease enzyme. And this fact — acknowledging the role played by nucleases in growth regulation — draws attention to a new aspect of the relation between zinc and growth regulation — beside the hormonal control of nucleases.

Considering the great importance of the practical, agricultural aspects of zinc supply, further researches carried out at a deeper, biochemical level on the biological effects of zinc are by all means necessary. So, for example, it is important to find out which component of nuclease it is that zinc acts on, how this takes place in vivo, and how it leads to growth stimulations or to the maintenance of normal morphological conditions, and later the regulation of senescence processes too.

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COMPARATIVE EVALUATION OF THE HATCHABILITY OF SINGLE- AND THREE-WAY CROSS COMBINATIONS IN MEAT TYPE FOWL

By

P. HORN

AGRICULTURAL COLLEGE, DEPARTMENT OF POULTRY AND
SMALL ANIMAL BREEDING, KAPOSVÁR

By studying the hatchability of three single- and six three-way cross broiler combinations in an experiment arranged in random blocks with 11 replications it has been established that the fertility of line crossed white Plymouth mother stocks considerably exceeds that of mothers belonging to pure lines. Crossed mothers produced 2.62 per cent more fertile eggs on an average which means a relative superiority of 23 per cent. Vitality at the embryonal stage of the broilers in the three-way cross broile combinations improved significantly too. 5.64 per cent of the embryos of single cross broilers and 4.89 per cent of the three-way cross broilers died in the first six days of hatching. The relative superiority of three-way cross combinations was 13 per cent. It was decisively due to a better fertility and higher vitality at the early embryonal stage that the hatchability of three-way cross broiler combinations — as calculated on the basis of the total number of eggs set — increased by an average of 2.83 per cent too. When the egg producing parent stocks or the embryos developing in the eggs were subjected to unfavourable environmental effects, the three-way cross combinations proved even more superior to the single cross ones regarding both the embryonal mortality and hatchability calculated as a percentage of the number of eggs placed in the incubator. In contrast to the general tendencies some combinations showed a higher degree and significant heterosis. Among combinations reciprocal on the mother's side no significant differences could be found.

Introduction

Hatchability can be best improved by the use of various cross-breeding methods, because only a very modest progress can be expected from selection within populations due to the low heritability of hatchability. A marked improvement of hatchability has been demonstrated in cross-breeding experiments using pure lines and various breeds.

Heterosis regarding hatchability has been reported — among others — by GLAZENER *et al.* (1952) evaluating Rhode Island Red, Barred Plymouth Rock, New Hampshire and White Leghorn crosses, by HORN *et al.* (1953) when crossing Rhode Island Red, Speckled and Yellow Hungarian and white Plymouth breeds, by HUTT—COLE (1952), COLE—HUTT (1962) crossing white Leghorn lines, NORDSKOG—GHOSTLEY (1954) crossing Australorp-, Rhode Island Red-, and New Hampshire breeds; by KRZANOWSKA (1959) crossing white Leghorn lines; by MERRITT—GOWE (1960) with inbred Plymouth lines, by HYRE—MCCLUNG (1965) with Rhode IslandRed-, New Hampshire- and white

Leghorn breeds; by TAGGE (1966) with white Leghorn lines, by STAMER (1967) with white Plymouth inbred lines; by ABRAHMANOVA (1968) with Zagorski-, Jubilee- and Cornish breeds, and by VINOGRADOVA (1969) with white Plymouth Rock-, New Hampshire-, Pervomajszkij and Adler breeds.

On the basis of the above cited investigations it could be established, that crossing lines or breeds influenced hatchability favourably. In many cases cross-bred stocks showed a higher hatching percentage than even the better parent. Significant differences were found, however, regarding hatchability between the individual combinations and reciprocal crosses.

The hatchability of meat type hen populations has been studied, by relatively few authors, as seen from the literature cited, although poor hatchability generally characteristic of meat type hen populations means a far greater economic problem than in the case of either dual purpose or layer type stocks.

Our investigations were aimed at finding out to what extent the hatchability of broilers changed depending on whether the mothers were hens belonging to pure-bred white Plymouth lines or they themselves originated from the crossing of two white Plymouth lines.

In the case of the single cross broiler combinations included in the study the paternal partner was in each case a cock of white Cornish breed, while the mother a hen belonging to a definite line of white Plymouth breed. In the case of the three-way cross combinations the paternal partner was a cock belonging to the white Cornish breed, but the mother originated from the crossing of two white Plymouth lines.

Material and Method

For the purpose of the investigation — as maternal crossing partners — line bred white Plymouth lines marked A, B and C, as well as all possible white Plymouth × white Plymouth cross combinations of these three lines were available at the Research Institute of Small Animal Breeding, Gödöllő.

As paternal crossing partners, cocks belonging to a definite line of the white Cornish breed were used to produce all single- and three-way cross broiler combinations.

The difference in hatchability of the various broiler combinations was thus caused by the genotypic differences of the mothers and the broilers only, since the male partner belonged to the same Cornish line in every cases.

Intensive selection has been carried out since 1965 to improve the early growth rate and slaughtering qualities in the different maternal and paternal lines. The coefficient of inbreeding did not exceed 0.7 per cent in any of the generations (SZIGETI 1972). All stocks included in the study were hatched at the same time and reared intermingled in order to make the environmental factors as uniform as possible for all the stocks tested. All experimental stocks reached sexual maturity practically at the same age, reaching 50 per cent production between 205 and 208 days of age (HORN 1971).

Taking into consideration that hatchability is influenced by the most divergent environmental factors — which affect not only the laying hen, but also the embryo developing in the egg — the repeatability of hatching results is extremely low and the experimental error may be considerable. Therefore the hatching experiment was carried out in a block design with 11 replications. To reduce experimental error the variancies caused by the age of the various parent stocks, the conditions of egg storage, and the effect of the incubators used (which affected all stocks tested uniformly) could be summarized in the block (replication) effect. Eggs were set into incubators every month from the middle of January to the end of September 1969, twice in January and April, and once in the other months. In each case the eggs were stored for seven days. The collected eggs were not selected because of methodological considerations with the exception of cracked or broken eggs.

In order to investigate the differences in hatchability between single and three-way cross broiler combinations the following parameters were assessed: 1. Average hatchability based on the total number of eggs placed in the incubator (%), 2. Average hatchability based on the number of fertile eggs (%), 3. Average percentage of infertile eggs (%), 4. Average percentage of eggs with blood rings (%), 5. Percentage of dead embryos between the first and second candling (%), 6. Percentage of choked chicks (%), 7. Percentage of poor quality chicks (%).

(In points 4—7 percentage is understood relative to the number of fertile eggs.)

The average hatchability was in each case determined on the basis of the number of first class chicks. The criteria of first class baby chicks were identical with those described by Kiss (1968).

Hatching was carried out in GX 10.000 type incubators. Candling was performed on the seventh and seventeenth day of hatching. The hatching technology corresponded in every respect to the technology employed at the Research Institute of Small Animal Breeding, Gödöllő.

Table 1 summarizes the data of the parent stocks included in the experiment, the total number of eggs placed in the incubators during the experiment and the number of fertile eggs, as well as the number of first class baby chicks hatched.

Table 1

Initial number of the different parent partner stocks; number of eggs placed in the incubator during the experiment; number of fertile eggs; number of baby chicks hatched

(Hatching experiment with 11 replications, Gödöllő, January—September, 1969)

Parent partners				Number of eggs placed in the incubator	Number of fertile eggs	Number of baby chicks hatched
Father		Mother				
Designation	Number	Designation	Number			
Single cross combinations						
Cornish ♂	16	A(Plymouth) ♀	162	4527	3975	3214
Cornish ♂	12	B(Plymouth) ♀	123	3487	3081	2539
Cornish ♂	16	C(Plymouth) ♀	162	4383	4038	3349
Three-way cross combinations						
Cornish ♂	14	AB ♀	141	4126	3853	3130
Cornish ♂	16	BA ♀	162	4719	4351	3620
Cornish ♂	16	AC ♀	157	4040	3702	3077
Cornish ♂	16	CA ♀	165	4737	4436	3597
Cornish ♂	16	BC ♀	160	3480	3212	2614
Cornish ♂	11	CB ♀	114	4208	3858	3174
Total:	133		1346	37707	34506	28314

Results

When evaluating the hatchability of single- and three-way cross broiler combinations significant differences — of practical importance too — were found between the individual combinations concerning the percentage of infertile eggs and of those having blood rings and the average hatchability related to the number of total eggs set (Table 2).

We did not succeed on the other hand in finding significant differences between the various broiler combinations — even with 10 per cent probability of error — regarding the following parameters: 1. Percentage of dead embryos between the first and second candling, 2. Percentage of chicks dead in the shell, 3. Percentage of poor quality and cull chicks, 4. Average percentage of hatchability based on the number of fertile eggs.

Table 2

Mean values of parameters determining the hatchability of various single- and three-way cross broiler combinations

Broiler combinations	Parameters determining the hatchability		
	Proportion of infertile eggs (%)	Proportion of eggs with blood rings (%)	Hatchability relative to the number of eggs placed in the incubator (%)
Single cross broiler combinations:			
Cornish ♂ × A ♀	12.28	6.64	70.63
Cornish ♂ × B ♀	12.62	5.26	71.86
Cornish ♂ × C ♀	8.09	5.03	75.02
Three-way cross broiler combinations:			
Cornish ♂ × AB ♀	7.14	4.29	75.96
Cornish ♂ × BA ♀	8.01	5.08	76.12
Cornish ♂ × AC ♀	8.20	4.71	75.41
Cornish ♂ × CA ♀	8.70	5.35	75.45
Cornish ♂ × BC ♀	9.76	5.10	74.03
Cornish ♂ × CB ♀	8.67	4.85	75.01
L.S.D.$_{5\%}$	2.20	0.99	2.93
L.S.D.$_{1\%}$	2.93	1.32	3.52

Of the four parameters listed above no tables are given. In Table 2 only those parameters are included in which there were significant differences between the tested combinations.

Table 2 shows the mean values of the hatching parameters of the various cross combinations. Table 3 summarize the analyses of variance, which were calculated to test the reliability of the differences between the mean values. Regarding the percentage of infertile eggs highly significant ($P < 0.001$) differences were found between the various combinations. The ratio of infertile eggs was relatively high in the case of white Plymouth hens belonging to the pure lines marked A and B. Line C was significantly more fertile than lines A and B. The fertility of the various cross-bred white Plymouth hens (AB and BA etc.) exceeded in each case the average of the corresponding parents.

Among the eggs of the cross-bred hens marked AB and BA the number of fertile eggs was 5.14 and 4.27 per cent more respectively than in the case of the better parental pure line. The differences were highly significant ($P < 0.01$).

Table 3

Summarization of variance analyses of the results of the hatching experiment

Factors	Parameters determining hatchability								
	Proportion of sterile eggs			Proportion of eggs with blood rings			Hatchability relative to the number of eggs placed in the incubator		
	SQ %	FG	F	SQ %	FG	F	SQ %	FG	F
Replication	56	10		57	10		52	10	
Crossing	20	8	9.09***	11	8	3.25**	12	8	3.13**
Error	24	80		32	80		36	80	

** $P < 0.01$
*** $P < 0.001$

In the case of the combinations developed with the white Plymouth line C the heterotic effect was not so marked as in the previously mentioned combinations, but the average fertility of the cross-breds was in every case closer to that of the better parent marked C.

No significant differences could be demonstrated, however, between the reciprocal combinations.

The percentage of eggs with blood rings as determined by candling on the seventh day of hatching reflects the degree of early embryonic mortality.

The most unfavourable result regarding the percentage of eggs with blood rings was shown by the single cross combination Cornish ♂ × A ♀. This combination was also significantly worse than either of the two single cross combinations included in the experiment.

A considerable heterosis effect was observed in the vitality of the three-way cross broiler embryos originating from the cross-bred AB and BA hens and Cornish cocks up to the seventh day of incubation, and they even exceeded the result of the better control single cross combination (Cornish ♂ × B ♀).

In the case of the Cornish ♂×BA ♀ three-way cross combination this difference reached significance at a 5 per cent probability of error.

The extent of average heterosis regarding blood ringed eggs can be demonstrated by the fact that in the single cross broiler combinations originating from the white Plymouth female lines marked A, B, and C 5.64 per cent of the embryos died up to the seventh day of incubation, whereas the corresponding figure is only 4.89 per cent in the case of the three-way cross combinations.

Between the three-way cross combinations reciprocal at the mothers' side there were no significant differences in the average percentage of the blood ringed eggs.

The degree of hatchability relative to the number of eggs placed in the incubator is practically the most important parameter of hatchability in the case of the broiler parent stocks. As for hatchability relative to the number of eggs placed in the incubator considerable differences were shown between combinations. Eggs produced by the Plymouth mothers A and B were of relatively poor average hatchability. The hatchability of eggs originating from the Plymouth stock C was found to be significantly better than that of the former ones. ($P < 0.01$).

Combinations where the mother had already been line crossed distinguished themselves by a much better hatchability. An expressed heterosis effect was experienced in the case of combinations Cornish ♂×AB ♀ and Cornish ♂×BA ♀ respectively, in both cases hatchability improved by more than 4 per cent even when compared to the better control combination (Cornish ♂×B ♀). The differences were significant ($P < 0.01$). A heterosis effect was shown in the other three-way cross combinations too, as the average hatchability was considerably better in all the three-way cross combinations than in the corresponding single cross combinations. No significant differences in hatchability relative to the number of eggs placed in the incubator were found between the combinations reciprocal at the mother's side.

Discussion

By studying the parameters determining the hatchability of single and three-way cross broiler combinations it has been established that in a breeding program aimed at producing highly productive broiler parents hatchability can be greatly improved by developing line-crossed white Plymouth stocks as maternal parent partners. It was especially remarkable that the fertility of line-crossed white Plymouth mothers showed a considerable degree of heterosis.

Among the eggs of line-crossed hens on an average only 8.41 per cent were infertile taking every combination into consideration —, while in the case of hens belonging to pure lines 11.03 per cent. The difference in favour of the crossed mothers is 2.62 per cent, which corresponds to a relative superiority of 23 per cent.

A similarly important general heterosis effect was demonstrable concerning reduced mortality at the early embryonal stage in favour of the three-way cross broiler combinations, since in the case of single cross broiler combinations an average of 5.64 per cent, in the three-way cross broiler combinations only 4.89 per cent of the embryos died before the seventh day of incubation. The relative superiority of the three-way cross combinations to single cross ones was 13.2 per cent.

In the three-way cross combinations the improvement of hatchability based on the total number of eggs set can mainly be explained by the increased fertility of the cross-bred broiler type mothers and the higher vitality of the three-way cross broiler embryos. A higher embryonic vitality could only be proved, however, at the early stages of incubation, as after the seventh day of incubation no significant differences caused by genetic factors could be demonstrated between the various cross combinations.

In connection with the observed improvement in fertility due to crossing meat type fowl lines, an interesting comparison can be made between the results of a number of cross-breeding experiments performed with turkey breeds and laboratory animal species on one hand and our own experimental results on the other.

The major role of the female genotype in determining infertility and the extent of early embryonic mortality was pointed out by McCarthy and Martin *et al.* (cit. GRAVERT 1969). The authors checked the development of fertilized egg cells in the periods preceding inplantation in laboratory animals. They found that the frequency of ovulation was equal in the case of inbred and cross-bred females. 64 per cent of the fertilized egg cells were not implanted in those cases in which inbred males and females were mated; the zygotes died at the moment of fertilization. When inbred females were mated with males belonging to different lines 42 per cent of the egg cells fertilized were not inplanted. When, however, the mothers themselves and the embryos too were cross-breds, the egg cells were inplanted to 100 per cent.

MCCARTNEY—CHAMBERLIN (1961) FRIARS *et al.* (1963) found in turkeys that the fertility of pure-bred hens did not improve when they had been mated to males belonging to a different breed. A considerable improvement occurred, however, when the hens themselves were already cross-breds. The genotype of the zygote did not influence fertility. In the turkey experiment cited, the relative importance of the early embryonic mortality affecting the measure of infertility could not be determined. A considerable experimental error may be included when determining the number and the percentage of infertile eggs by candling on the 7th day of incubation. In the case of zygotes at the moment of fertilization or shortly after, the corresponding egg is considered infertile in several cases with a high probability, as no visible blood rings are apparent. The problem of misjudgement is even more frequent in the case of eggs with a shell of darker colour. By studying Leghorn eggs KRZANOWSKA (1959) arrived at the conclusion that the first peak of embryonic mortality occurred immediately after fertilization, and in these cases the eggs were considered sterile. Similar experimental results are listed by HUNTON (1969). The mentioned sources of error indicate that the percentage of infertile eggs of the various cross combinations are overestimated in our experiments too, the percentage of blood ringed eggs, however, only gives an underestimated measure of the true incidence of early embryonic mortality (HORN 1972). The latter assumption is confirmed to a certain extent by the fact, that in the case of several cross combinations included in this study, a certain correlation exists between the percentage of infertile eggs and that of blood ringed eggs inasmuch, as a greater extent of infertility is accompanied by a higher percentage of eggs with blood rings and vice versa.

The experimental results cited and our own experiments suggest that the female plays a very important role in determining the fertility and early embryonal mortality of the offspring. In broiler production a considerable improvement of hatchability can be expected from line-cross-bred mothers and closely connected with this, from three-way broiler cross combinations.

From numerous studies and practical experience it is well known that during the egg production the hatchability of eggs is highly varying. In the initial period following the beginning of egg production hatchability is usually poor, then a gradual improvement can be observed; while towards the end of the egg producing season hatchability decreases again. Fig. 1 compares the percentage of infertile eggs generally characteristic of white Plymouth mothers belonging to pure lines (A, B, C) — in monthly intervals — with the average percentage of the six line-crossed mother stocks. Fig. 1 and Fig. 2 illustrate the percentage proportion of eggs with blood-rings in a similar way. Fig. 3 shows the hatchability averages of single and three-way cross broiler combinations, relative to number of eggs placed in the incubator.

Fig. 1 shows that the average infertility of mothers belonging to pure lines was always inferior to that of cross-bred mothers throughout the whole period of egg production. The superiority of cross-bred hens was apparent during the entire experimental period. Fig 2.

Fig. 2 reflects an interesting phenomenon. The percentage of eggs with blood-rings shows changes of a definite tendency during the period of egg production in this case too, but it is highly remarkable that in January, February, July and August, — when a greater extent of embryonal mortality occurred — the superiority of three-way cross broilers originating from cross-bred mothers compared to single cross combinations increased considerably. In March, April, May and June — taking the averages into consideration — no practical

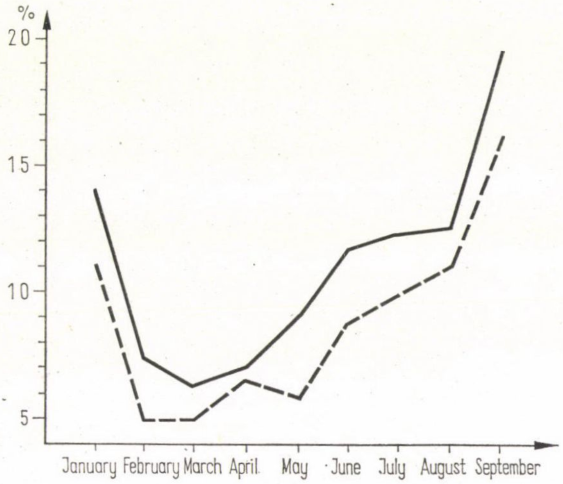

Fig. 1. **Monthly averages (%) of infertile eggs of white Plymouth hens belonging to pure-bred lines, and of line-crossed white Plymouth hens (———— = hens belonging to pure-bred lines; — — — — — = line-crossed hens)**

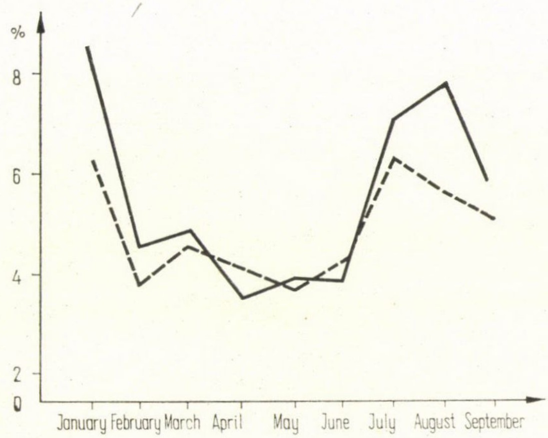

Fig. 2. **Monthly averages (%) of eggs with blood-rings in single and three-way cross broiler combinations (——— = single cross broiler combinations; - - - - - = three-way cross broiler combinations)**

difference was found in the degree of early embryonal mortality. When the environmental conditions — which affected the layers, the eggs stored and through them the embryos alike — did not essentially deviate from the optimum, the embryonal vitality of the three-way cross broilers did not show general heterosis effects compared to the single cross broilers. When, however, the embryos were exposed to harmful effects, the superiority of three-way cross broilers regarding embryo vitality became apparent. Such damaging factors may have been e.g. various qualitative deficiencies of eggs after the beginning of egg production (January), or a higher than optimum temperature of the egg storing room, which often exceeded 24 °C in July and August. Fig. 3.

Similar tendencies are reflected in Fig. 3. regarding hatchability relative to the number of eggs placed in the incubator, inasmuch as the superiority of three-way cross combinations in this respect is more expressed in the first two and last three months of egg production than in the period between.

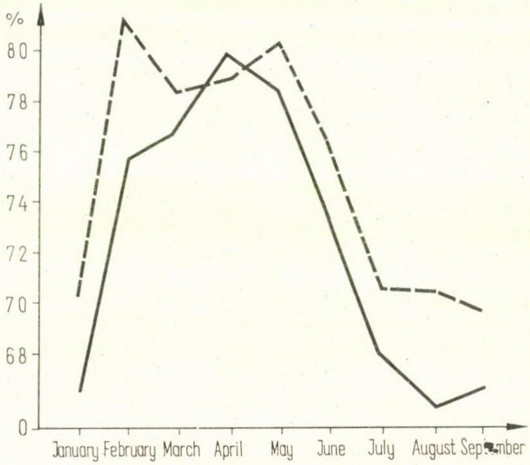

Fig. 3. Average hatchability (%) of single- and three-way cross broiler combinations as related to the number of eggs placed in the incubator, in a monthly distribution (——— = single cross broiler combinations; - - - - - = three-way cross broiler combinations)

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DIAPAUSE OF HYPHANTRIA CUNEA DRURY (LEPID.: ARCTIIDAE) IN HUNGARY

By

Gy. Sáringer

LABORATORY OF THE RESEARCH INSTITUTE FOR PLANT PROTECTION, KESZTHELY

The diapause of *H. cunea* in pupation is determined at a temperature of 18 and 23 °C primarily by the photoperiod. Higher temperatures (28 °C) decrease the effect of the photoperiod. The critical time of illumination that induces diapause is between 14 and 15 hours. The quality of the host plant also has some effect on the appearance of diapause. Changing temperature (outdoor in the year 1970) did not modify the photoperiod induced diapause. Illumination conditions are perceived by the larvae in the second half of their development period. The size of the moth population in the second summer generation and of the larva population in the incomplete third generation depends to a great extent on the temperature. It is in years of high average temperature that major damages may occur.

Introduction

The biology of the fall webworm (*Hyphantria cunea*) was cleared up within ten years following its appearance (1940) in Hungary (Issekutz 1946, Surányi 1946, 1947, 1948, Kadocsa 1946, Jermy 1948, 1952, Szelényi 1949, Szelényi—Viktorin 1948, Reichart 1951, 1952, Reichart—Szabó 1951, Nagy 1952a,b, 1953, Nagy *et al.* 1953, Szalay-Marzsó 1955, Gere 1956a,b, etc.). With the gradual spreading of the pest in Central Europe, more and more new data were published on the pest in the affected countries (Petrik 1951, Jasič—Birová 1958, Jasič—Macko 1961, Jasič *et al.* 1964). Investigation results based on experiments concerning the dormant state of this pest were first published by Jermy—Sáringer (1955). Later the effect of temperature, photoperiod and host plant quality on diapause in pupation was studied in a detailed experiment series by Jasič (1960), then by Jasič—Macko (1961) in Czechoslovakia.

We have already explained in our paper mentioned above (Jermy—Sáringer 1955) that diapause setting in during pupation is determined by the photoperiodic conditions prevailing during the larval development. In these investigation we found that the time of illumination critical for the diapause was a daily photoperiod of 14/10 hours (September, glasshouse-laboratory). The investigations were made with the larva progeny of the first summer generation. In the course of the experiments we pointed out, further, that the photosensitive phase of development was in the second half of the period of larval development, and that the quality of the host plant (*Morus alba* leaf) did not influence the diapause.

Results obtained by JASIČ (1960) and JASIČ—MACKO (1961) can be summed up shortly as follows: 1. Pupae developing from larvae raised at a high temperature (28 °C) and in full darkness showed a very low percentage diapause. When raising took place at temperatures below 22 °C, again in full darkness, the diapause was above 90 per cent. There was a difference found in the diapause percentage between the first and second generations. At the same temperature the percentage of diapause was always higher in the second generation. From this the authors drew the conclusion that the age of the host plant also played a part in inducing the diapause. From larvae raised at 18 °C and in full darkness only diapausing pupae developed in both generations. 2. At temperatures of 21—22 °C the critical time of illumination was determined as a daily photoperiod of 14/10 hours. The ecological requirements of two summer generations are met under the geographical conditions of Czechoslovakia. 3. Under the authors' experimental conditions (21 and 22—25 °C respectively, long-day during the development of L_1 and $L_1 - L_2$, daily photoperiod of 18/6 hours, then full darkness) it was in the first half of larval development that the larvae perceived the illumination conditions critical for the diapause. This statement of theirs contradicts the results obtained by us, so later we shall return to this subject.

On the outdoor diapause of the first summer generation data were already published by NAGY *et al.* (1953). According to the latter authors in 1951 1.14 per cent of the pupae in the first generation remained in diapause (on the basis of 18,600 pupae from 19 sites of Hungary). From a part of the diapausing pupae moths hatched in September—October, from another part in the following spring or at the beginning of the following summer. In the second generation 11.3 per cent dormant pupae were found. According to the authors' observations pupae in diapause do not require the effect of low temperature to reactivate, since when kept at room temperature, after 5 months of dormant state the moths began to fly. From such pupae the hatching of moths lasted more than five months.

In order to ensure a better understanding of our results, before discussing in detail our own experiments we shall summarize briefly the development of *H. cunea* in Hungary on the basis of the work of NAGY *et al.* (1953) and of our own phenological investigations made in the district of Keszthely (South-West Transdanubia).

The swarming of moths from the overwintering (diapausing) pupae generally starts in April—May. The egg laying period of the overwintering generation is between the middle of April and middle of May. Caterpillars' nests can be seen as early as the first days of May. Damages are done by the caterpillars until the middle of July, but pupation already begins in the second half of

June. Pupae of the first generation can be found even in the middle of August.

The swarming of moths from the first summer generation lasts from early July to the end of August. The larva progeny of this generation may do damages from the middle of July up to the end of September. Pupation begins at the end of August. The great majority of the pupae diapause and overwinter. In favourable seasons (late summer with high temperatures) from the low number of pupae not entering a diapause the swarming of moths begins from early September. This small moth population of the second summer generation lays eggs. The larvae can be found till late autumn (fall of leaves), but due to the cold autumn weather die before pupation. This generation is called the third, incomplete generation.

By studying the diapause conditions of *H. cunea* we wished to throw light on the causes of differences between the results of our pilot experiments performed in the middle of the fifties (JERMY—SÁRINGER 1955) and the above results of JASIČ—MACKO (1961), then in possession of the results explain the phenology of the generations. With this in view our investigations were performed according to the following aspects:

1. Role of various constant temperatures and photoperiods in inducing the dormant state of pupae.
2. Effect of changing temperature and various photoperiods on the diapause induction of pupae.
3. Determination of the photosensitive phase of development.
4. Explanation of the appearance of the incomplete summer generation III in Hungary.

Material and Method

The pupa population used as initial material was collected in the autumn of 1969 in the neighbourhood of Keszthely. Pupae overwintered in a bundle of corrugated paper placed in an outdoor insectary. Imagos swarming out in the spring of 1970 were placed on mulberry (*Morus alba*) shoots in a wooden-frame cage covered with a wire screen, where they laid eggs. The eggs were kept in an aqueous hygrostate during the embryonal development. The larvae were raised in three thermostates of constant temperature (28 ± 0.7, 23 ± 1.2 and 18 ± 1.3 °C) with five different periods of illumination (daily photoperiods of 13/11, 14/10, 15/9, 16/8 and 17/7 hours). Light intensity ranged between 280 and 300 lux. The culture pot was a net covered glass cylinder of 24 cm height and 11 cm diameter, in which the mulberry (*Morus alba*) shoot was placed in a water-bottle. For the purpose of pupation a bundle of corrugated paper rolled up loosely was placed in the culture pots. In each illumination treatment 180—250 eggs were used in the experiments. While raising the larvae we found a 42—71 per cent mortality.

The evaluation of the diapause stage was carried out after a sixty days dormant state of the pupae.

The photosensitive phase of development was determined in a chamber of 23 ± 1.2 °C by placing one group of cultures prepared from a total of 150 eggs laid within 24 hours under long-day conditions (a daily photoperiod of 17/7 hours), and the other group of cultures containing the same amount of eggs under short-day conditions (a daily photoperiod of 13/11 hours). Knowing the average time of larval development we interchanged the cultures at half-time. The experiment was performed with larvae of the first summer generation in June—July 1970.

Experiments carried out with a changing temperature and different photoperiods took place in an open air insectary. During the development of the first summer generation (1 June—16 July 1970) the average temperature was 20.3 °C, while at the time of the second summer generation (21 July—19 September) 19.6 °C.

Results

1. Effect of various constant temperatures and photoperiods on the dormant state of pupae. Diapause curves obtained with three constant temperatures and 5 photoperiods each for generations I and II are shown in Fig. 1. Concerning the individual generations the following can be established: at temperatures of 18 and 23 °C the diapause of pupae is primarily induced by the photoperiodic conditions prevailing during the larval development. Higher temperatures (28 °C) influence the diapause induction of the photoperiod by decreasing its effect. As a result diapause falls below fifty per cent even on short days. The effect of temperature on the diapause can also be observed between 18 and 23 °C.

The role of temperature in modifying the diapause percentages can be observed in the case of both generations.

The quality of the host plant also plays a part in inducing the diapause of the pupae. At the time of the experiments performed with the second generation the larvae fed on older leaves which resulted in higher percentages of diapause. Since at the time of the experiment all external factors agreed with those of the first generation, the higher diapause percentages can be explained by qualitative changes in the host plant. Thus, on the basis of the latter experiments our relevant statements published earlier (JERMY—SÁRINGER 1955) must be revised. Our results concerning the effect of the quality of the host plant on the diapause confirm those obtained by JASIČ—MACKO (1961).

On the basis of the experiments the time of illumination critical for the diapause is between 14 and 15 hours. Similar results are found in our paper published in 1955 as well as in the work of JASIČ—MACKO (1961).

It is seen from the curves of Fig. 1 that at temperatures of 18 and 23 °C a few per cent of diapause occurred even on long days (with daily photoperiods of 16/8 and 17/7 hours). In generation II this was found in a higher percentage. This phenomenon confirms the observation made by NAGY *et al.* (1953) namely, that under natural conditions a 1.14 per cent diapause occurred in the first generation.

2. Effect of a changing temperature and different photoperiods on diapause induction. The results of experiments performed in the outdoor insectary with six periods of illumination are presented in Fig. 2. According to the trends of the curves the changing temperature did not modify the photoperiodically induced diapause. The higher diapause percentages of the second summer gener-

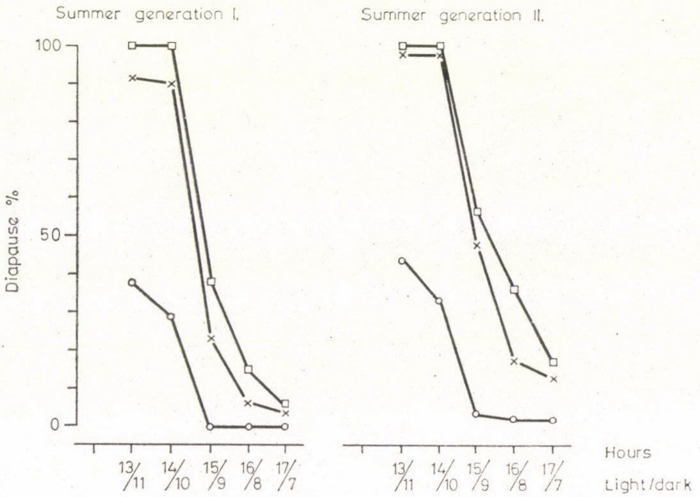

Fig. 1. Diapause curves of *Hyphantria cunea* at three temperatures and in five photoperiods. □——□ = 18 °C, x——x = 23 °C, o——o = 28 °C

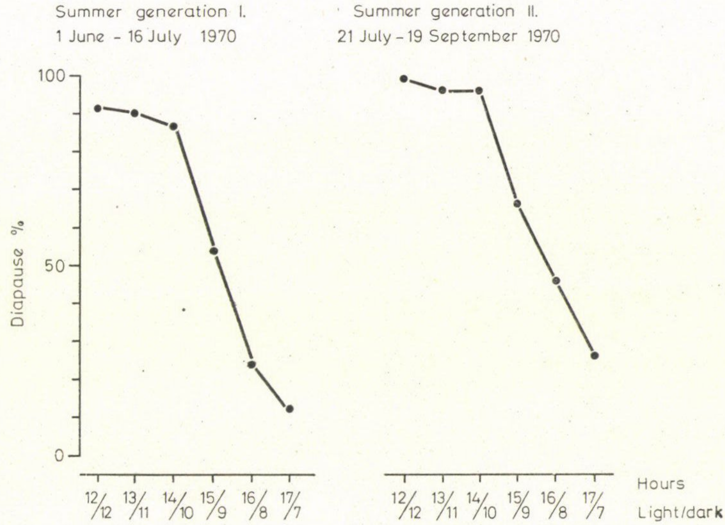

Fig. 2. Diapause curves of two summer generations of *Hyphantria cunea* under changing temperature conditions (in an outdoor insectary)

ation compared to generation I also prove the effect of the host plant quality on the increase of the diapause.

3. Determination of the photosensitive phase of development. Since in our pilot experiments (JERMY—SÁRINGER 1955) photoperiodical conditions critical for the diapause were perceived by larvae in a later stage of development, while according to JASIČ—MACKÓ (1961) by those in the first and second

stage of development, it seemed necessary to study the question more closely. Results are given subsequently:

First half of larval development:	short-day,	13/11	diapause 11%
Second half of larval development:	long-day,	17/7	
First half of larval development:	long-day,	17/7	diapause 100%
Second half of larval development:	short-day,	13/11	

The above data confirm our statement made in 1955 and cited before, namely, that only the older larvae are responsive to the duration of illumination. In this respect the results obtained by Jasič—Macko (1961) must be considered incorrect, since, after exposing the larvae at stages L_1 and L_2 to long-day illumination, they ensured full darkness in the subsequent phase of larval development.

4. How can the appearance in Hungary of the incomplete generation III. be explained? Knowing the diapause conditions of *H. cunea* we shall try to outline the circumstances that make the appearance in Hungary of the incomplete generation III. possible.

The results of investigations show that within a certain range of temperature (18—23 °C) the diapause of pupae primarily depends on the photoperiod. High temperatures (28 °C), on the other hand, to some extent prevent the development of diapausing pupae, irrespective of the photoperiod. The critical time of illumination is a daily photoperiod of 14/10 hours. In the district of Keszthely the effective day-length between 20 April and 21 August is more than 14 hours. Knowing the annual course of development of *H. cunea* and the photoperiodic conditions precisely we should say that in Hungary ecological conditions are satisfactory for the development of two summer generations. The fact, that the pupae of the second summer generation do not show a 100 per cent diapause after all, can be explained by the earlier swarming and egg laying of the moths in springs with high temperatures. If the summer is also hot, the development of the larvae is also more rapid, consequently the moths of the first summer generation swarm earlier again, and a higher proportion of their larva progenies develops on days longer than 14 hours. Thus the possibility is given that pupae originating from them will continue their development without a diapause. But even the pupae originating from larvae developing on days shorter than the critical time of illumination will not show diapause if a higher temperature (28 °C) prevails during the development. Since temperature conditions vary with years and seasons it may occur that a larger larva population of the incomplete third generation can be found in one year than in another.

The time when the generations — especially the moths of the second summer and larvae of the incomplete third generation — appear is decisively influenced by the temperature conditions of the respective year.

According to our investigations, pupae that have developed from larvae raised under long-day conditions, and do not enter diapause are not able to overwinter.

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HAY AND LEAF MEALS OF SOYBEAN AND SUNFLOWER UTILIZED IN BACON PIGS

By

E. Kurnik, D. Gábos, B. I. Pozsár

AGRICULTURAL RESEARCH INSTITUTE, IREGSZEMCSE;
AGRICULTURAL TECHNICAL SCHOOL, CSORNA; INSTITUTE OF AGROBOTANY, TÁPIÓSZELE

Authors used three groups of large white bacon type pigs in a feeding experiment to determine the biological efficiency of hay meals and leaf meals of sunflower and soybean. According to the results of the 70 and 50 days experiments carried out with 15 porklings each, fodder supplemented with a 1 : 1 ratio mixture of sunflower hay and leaf meal, respectively, was consumed after one or two days of reluctance while the similar mixture of soya hay and leaf meal willingly at once by the animals. From the composition of the rations it was seen that they consumed five per cent of their body weight to the full. The production data show that the protein contents of sunflower and soya meals — though they cannot be regarded as concentrated — substituted for the fodder protein in the case of fodder barley. The average daily weight gain of the three groups was 630 g followed by an average of 622 g attained with sunflower meal fed; at the same time soya exceeded the control (634 g) with its 668 g daily average weight gain. This latter, too, proves that it is just by its excellent range of amino acids that soya protein ensures a higher biological efficiency. Porklings included in the experiment reached a live weight of 65—70 kg within four or four and a half months. The data of weight gain show that the daily weight gain of the animals varies in a relatively wide interval for which the reasons are unknown for the time being.

Introduction

In an earlier publication authors reported on results according to which the pulped soybean silage had an increasing effect on milk production in various phases of the lactation period (Kurnik—Gábos—Pozsár 1961). The relatively large protein content of the soybean as well as the outstandingly high level of limiting essential amino acids (lysine, methionine) compared to the proteins of plant origin and even to the papilionaceous fodder plants (Spector 1956) made the excellent biological efficiency probable. On the other hand, it is today a basic principle of protein management that leaf proteins are increasingly used for the replacement of seed proteins (grain fodders), and leaf proteins are concentrated by various technologies for feeding purposes and for the preparation of human nutrients, from dietetic aspects (Pirie 1969). Starting from this idea authors prepared leaf meals and studied their importance in feeding in relation to the weight gain of porklings. With the partial substitution of leaf meals made of sunflower and soybean authors attempted to prove that the daily feed rations can be made more diversified on one hand, and that from the point of view of production biology leaf proteins may increase the live weight to the same extent as seed proteins do.

Material and Method

In the feeding experiment large white bacon type pigs kept in the Csehi farm unit of the Experiment Farm of Iregszemcse were used. The experiments were started on 1st May with piglets of 26 kg average weight. The piglets were kept in two separate groups; group I.

Table 1

Feeding composition of leaf meals and hay meals of sunflower and soybean in percentage related to the dry matter

Components	Soybean		Sunflower	
	leaf meal	hay meal	leaf meal	hay meal
Water content	9.65	9.09	11.15	11.75
Crude protein	18.05	11.82	15.97	10.79
True protein	14.52	8.92	14.10	9.34
Raw fat	3.98	3.09	2.13	2.20
Raw fibre	17.32	23.55	6.32	14.62
Nitrogen-free extracts	37.19	33.92	46.85	53.34
Ashes	8.94	8.70	14.63	9.71
Starch value	47.93	42.63	46.80	52.02
Digestible protein	9.87	6.06	7.90	5.83

Table 2

Age and initial weight of porklings included in the sunflower and soybean hay and leaf meal feeding experiment, and weight gain during the two-weeks periods

Group	Experiment periods with weighing		Porklings			
	date	periods in days	age in months	total weight in kg	number of animals	average weight in kg
I	1 May—16 May	15	2.5	390	15	26.0
	16 May—1 June	16	3.0	513	15	34.2
	1 June—15 June	15	3.5	668	15	44.5
	15 June—1 July	15	4.0	775	15	51.7
	1 July—10 July	9	4.5	951	15	63.4
II	1 May—16 May	15	2.5	390	15	26.0
	16 May—1 June	16	3.0	522	15	34.8
	1 June—15 June	15	3.5	686	15	45.7
	15 June—1 July	15	4.0	819	15	54.6
	1 July—10 July	9	4.5	969	15	64.6
III	20 May—1 June	10	2.5	465	15	31.0
	1 June—15 June	15	3.0	551	15	36.7
	15 June—1 July	15	3.5	708	15	47.2
	1 July—10 July	10	4.0	869	15	57.9

Table 3

Percentage and kg values of daily rations in the different periods of the feeding experiment carried out with hay and leaf meals of sunflower and soybean

Group date		Percentage composition of daily rations								Skimmed milk kg	Other feed kg	Starch value	Digestible protein g
		barley grist	sunflower meal	soybean meal	maize grist	pig feed D_3 vit.	pea grist	peanut grist	linseed cake				
I	1 May	40	10		33	7	5	5		2	1.4	1.13	218
	16 May	35	10		40	10	5	7		2	1.5	1.19	247
	1 June	30	10		40	10			10	2	1.6	1.29	265
	15 June	30	10		40	10			10	2	1.8	1.46	273
	1 July	20	10		55	10			5	2	2.1	1.54	293
II	1 May	50			33	7	5	5		2	1.4	1.16	218
	16 May	45			33	7	10	5		2	1.5	1.26	249
	1 June	40			40	7	5	5		2	1.6	1.34	268
	15 June	40			40	10			10	2	1.8	1.49	273
	1 July	40			40	10			10	2	1.2	1.60	293
III	20 May	35		10	33	10	5	7		2	1.5	1.18	248
	1 June	30		10	40	10			10	2	1.6	1.27	266
	15 June	30		10	40	10			10	2	1.8	1.43	275
	1 July	10		10	55	10			5	2	2.1	1.54	297

was fed with hay and leaf meals of sunflower mixed at a ratio of 1 : 1, while group II was used as control; the protein demand of piglets was supplemented primarily with grain fodder protein; experimental study on group III began later with new accomodations put into operation on 20th May. Group III consisted of piglets with an average weight of 31 kg, so it could be given feed rations identical with those given to groups I and II, which made the comparison of production biological effectivity on development and live weight gain possible. Feeding experiments were carried on with 15 piglets in each group, in groups I and II for 70 days per each while in group III for 50 days. Group III was given hay and leaf meals of soybean in a mixture of 1 : 1 ratio.

Composition and feeding value of hay and leaf meals of sunflower and soybean are shown in Table 1.

At the beginning of the experiment the animals fed differently were placed in categories with their age and initial weight indicated, according to Table 2. The daily rations of the individual feeding groups are presented in Table 3. The amount, starch value and percentage digestible protein of feed given in the various phases of the experiment are given in kg in Table 4. The extent of weight gain during the period of experiment is shown in kg values by Table 5. with the exception of the data of daily weight gain (g). Starch value (kg) and digestible protein (g) required for the production of 1 kg live weight express the productive biological effectivity in relation with the leaf meals of sunflower and soybean, according to Table 6. Percentage utilization of starch value in the increase of live weight expresses, in fact, the effective carbohydrate recovery.

Table 4

Total kg weight of feed given in the different periods of the feeding experiment, and its starch value and digestible protein in kg

Group	Date	Feed in kg		
		Total amount	Starch value	Digestible protein content
I	1 May	21	16.9	3.27
	16 May	24	19.0	3.95
	1 June	24	19.3	3.97
	15 June	27	21.9	4.09
	1 July	19	13.8	2.63
II	1 May	21	17.4	3.27
	16 May	24	20.1	3.98
	1 June	24	20.1	4.02
	15 June	27	22.3	4.09
	1 July	19	14.4	2.63
III	20 May	15	11.8	2.48
	1 June	24	19.0	3.99
	15 June	27	21.5	4.12
	1 July	21	15.4	2.97

Results

Sunflower hay and leaf meals were produced by the quick drying and grinding of plants before flowering, while soya hay and leaf meals were made with the same procedures but of plants beginning to develop pods. Fodders with relatively high protein contents were included in the experiment on the basis of their starch value and crude protein content.

In the ration of group I the 10 per cent coarse barley meal was replaced by a sunflower hay and leaf meal mixture of 1 : 1 ratio. For one or two days pigs were averse to the sunflower meal probably because of the unusual glandular hairs. The ration of group III was completed to 10 per cent by a 1 : 1 mixture of soya hay and leaf meal instead of the coarse barley meal. Pigs consumed the soya meal always with good appetite.

According to the data presented in the tables animals in each group were given a daily amount of 2 kg skimmed milk per head, on the basis of the favourable results obtained in general. According to the data of Tables 3 and 4 the groups received every day the same starch value and digestible protein amounts for the very purpose of comparing the biological effectivity of sunflower and soybean meals.

Table 5

Production results in kg in the different periods of the feeding experiment; daily weight gain in g

Group	Date	Initial weight	Weight at the end	Average weight	Weight gained	Daily weight gain in g
I	1 May	390	513	34.2	8.2	546
	16 May	513	668	44.5	10.3	643
	1 June	668	775	54.7	7.2	480
	15 June	775	951	63.4	11.7	780
	1 July	951	1044	69.6	6.2	688
II	1 May	390	522	34.8	8.8	586
	16 May	522	668	45.7	10.9	681
	1 June	668	819	54.6	8.9	593
	15 June	819	969	64.6	10.0	666
	1 July	969	1056	70.4	5.8	644
III	20 May	465	551	36.7	5.7	570
	1 June	551	708	47.2	10.5	700
	15 June	708	869	57.9	10.7	713
	1 July	869	966	64.4	6.5	650
	Mean values:					
		Group I.			43.6	622
		Group II.			44.4	634
		Group III.			33.4	668

Table 6

Kg starch value used for the production of 1 kg live weight in the feeding experiment with hay and leaf meals of sunflower and soybean; g weight of digestible protein; percentage conversion of starch value

Group	Period in days	Starch value kg	Digestible protein g	Percentage conversion of starch value
I	70	2.08	410	48
II	70	2.12	405	47
III	50	2.02	406	49

Beside the comparison of feeding authors continually studied whether the rate of development and body weight increase in the individual groups would change. During the feeding experiments animals were weighed every second week so that the daily average weight increase could be computed, feed conversion capacity of animals in the different groups evaluated, with the fact that the genetic basis of weight increase was excellent, that is animals were able to consume every day with good appetite a feed quantity equal to or more than 5 per cent of their body weight, taken in consideration.

The initial weigh totals of groups I and II were identical; the average weight of the animals was 26 kg. Group III, on the other hand, started with a slightly higher (31 kg) average weight.

According to the data of Table 4 the feed rations were almost perfectly identical as to their starch value and digestible protein content.

Production results — as shown by Table 5 — did not decrease under the influence of sunflower and soybean meals fed; on the contrary, in the case of soybean they even increased. In this sense it can be safely accepted that the leaf protein of the two fodder plants studied is suitable to replace biologically the seed protein, in the present case the protein of the barley meal.

The total initial weight of 390 kg in the control group (II) increased during the 70 days period to 1056 kg. Accordingly, the average weight of animals increased from the initial average weight (26 kg) to 70.4 kg which means 44.4 kg weight surplus. In comparison, the average weight of animals fed with sunflower meal increased from 26 kg to 69.6 kg, which is an insignificant difference.

As a response to soybean meals fed body weight increased from an average of 31 kg to 64.4 kg during the 50 days period, which meant a 33.4 kg gain of weight.

Data on the daily gain of body weight reveal further that the average weight gain of the three groups included in the experiment is 630 g a day. In the group fed with sunflower meal it is lower (622 g), in the control group 634 g while in the group fed with soybean it is 668 g. Latter comparison shows that the amino acid proportion of the protein components of soybean is more favourable than that of the seed proteins of grain fodders.

The relatively high fluctuation (480—713 g) in the daily values of the outstanding average gain of body weight is difficult to explain, and the individual, ecological, etc. causes it can be traced back to as well as the possibilities of decreasing this fluctuation, are unknown for the present.

According to the data of Table 6 there is no significant difference between the groups in starch value required for the production of 1 kg live weight, proportion of digestible protein or percentage conversion of starch value. On this basis it can be stated that from the point of view of biological productivity the protein contents of hay meals and leaf meals are able to replace the seed proteins (grain fodder), moreover, as regards biological efficiency they even exceed them.

Discussion

According to the results of comparative feeding studies carried out with hay meals and leaf meals of sunflower and soybean leaf proteins are able to replace seed proteins as regards biological productivity. This finding is of great importance not only from the biological point view of production but also from feeding and farm management aspects, as it ensures the production of cheaper and larger volume feed per unit area.

It must be pointed out that the protein content of experimentally produced hay meals and leaf meals is still very low compared to the industrially produced leaf proteins with more than 40 per cent crude protein content made possible by quick drying, grinding then fractioning. On an experimental production level PIRIE (1969) extracted from leaf a concentrate with 75 per cent protein content, which can be used for human dietetic purposes, too. On the basis of preliminary studies it is highly probable that mechanically separated leaf meals with minimum fibre contents may be favourable basic materials of a protein concentrate which — supposedly — will compete with the seed proteins not only because of its economic advantages but due to its high biological productivity, too.

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FACTORS INFLUENCING THE QUANTITATIVE ANATOMICAL CHARACTERS OF THE VINE CANE II. STRUCTURE OF INTERNODES AT DIFFERENT LEVELS OF CANE

By

Á. HEGEDÜS

RESEARCH INSTITUTE FOR VITICULTURE AND ENOLOGY, BUDAPEST

The quantitative anatomical characters were studied in cross-sections excised from the middle of each internode of the mature vine cane. The sam measur ing, counting and ratio calculating was performed as published in our previous paper (HEGEDÜS 1967). The fluctuation of values from internode to internode was analysed as well as their increasing or decreasing nature along the cane. In a considerable proportion of the quantitative anatomical characters a definite increasing or decreasing tendency could be observed along the cane from the base to the apex, therefore if we wanted to demonstrate differences caused by the variety or by ecological effects, we had to take the samples from identical or nearly identical levels. As for the position of the examined internode to the tendrils no difference was found in the structural indices.

Introduction

In the first part of our study (HEGEDÜS 1967) the quantitative anatomical values of the vine canes were analysed according to vine, number of canes per vine, length of cane, length and thickness of internode in order to find out the method of sampling most suitable for a variety comparison. Now the results obtained when studying the canes in full length, by internodes are presented.

Material and Method

In order to determine the trends of the quantitative anatomical characters at the different levels of the cane we examined three intopped canes of each of the 5C (*Vitis Berlandieri* × *riparia*) and Portalis (*Vitis riparia*) varieties as well as one intopped cane of Olaszrizling (*Vitis vinifera)* at full length. The number of internodes was between 30 and 40 in each cane. With a view to the evaluation of the data it must be mentioned that on the third cane of 5C the shoot above the 12th node had broken off and the role of the main shoot was taken over by an axillary shoot. This fact had a decisive influence on the development of the inner structure in more than one respect. As to the Olaszrizling cane examined we have to note that the other shoots of the stake supported low cultivation vine were topped, while the one in question was tied to a nearly vertical wire and was exposed, consequently, to highly favourable light conditions, which — together with the omittance of topping — influenced its inner structure to a great extent. In addition to the structural indices we studied the length of the internode in each cane, and in the case of the Olaszrizling the thickness of the internode too.

The quantitative anatomical indices studied are: b: minimum diameter of pith; B: maximum diameter of pith; B/b: ratio of the two; f: minimum thickness of xylem; F: maximum thickness of xylem; F/f: ratio of the two; fb: wood-pith ratio according to the formula $\frac{2(f+F)}{b+B}$; h: minimum thickness of phloem; H: maximum thickness of phloem; H/h ratio of

the two; kh: average number of phloem-fiber layers; H/kh: proportion of total phloem thickness to the total thickness of phloem-fiber layers at the widest spot of the phloem; t: maximum diameter of trachea on the wide sides; T: maximum diameter of trachea on the narrow sides; T/t: ratio of the two former values; T_1/T_2: ratio of maximum trachea diameters at the ventral and dorsal side; bs_1: number of primary rays; bs_2: number of secondary rays; bs: total number of rays; 1/2: ratio of the numbers of primary and secondary rays.

Results

The twenty indices measured and calculated on the basis of the diameter of pith, thickness of xylem, thickness and structure of phloem, width of trachea and number of rays were plotted. For the sake of illustration we present the graph of the second cane of Portalis (Fig. 1). It can be seen in the figure which are the characters (b, B, F/f, H/h, H/kh) showing a relatively high fluctuation from internode to internode. The length of the internodes varies according to the position of the tendrils; internodes between two tendrils are the longest, but this variation is not reflected in either of the structural indices. On the seven canes examined we found that, in general, the internode between the two tendrils was the longest, and the one immediately above the former — that is limited below by a tendril and above by a bare node — the shortest. In the case of the canes studied this rule was effective in most cases, and in the following percentage: for the longest internode 93 per cent on the root-stock varieties and 67 per cent on the Olaszrizling, while for the shortest internode 87 per cent for the root-stock varieties and 56 per cent for the variety Olaszrizling. ZIMMERMANN (1954) points out that the different length of internodes has an effect on the inner structure too, therefore the canes have to be examined for maturity always by sections of three internodes.

This statement of Zimmermann is confirmed by the well-known experience that the shortest internode has the most favourable wood-pith ratio, though the question has not been systematically studied so far. In our present investigations we have found that the inner structural indices do not reflect the same periodical changes as clearly shown by the length of the internodes. The increasing or decreasing tendencies of the quantitative structural indices along the axis of the cane are also shown by the graph, and still better by Tables 1—5. In these tables the means of the 11—20 and 21—30 internodes of each cane were calculated and the least significant differences determined. There was no significant difference found in the maximum trachea diameter between the ventral and dorsal sides (T_1/T_2), while with the other values the F-test disclosed a very high significance. However, in the case of many quantitative data there was no significant difference found between the lower and upper cane levels; only the diferent canes showed significant differences. From the point of view of the two cane levels (internodes 11—20, and 21—30) the indices can be grouped according to the following:

values decreasing upwards: T, F, h, H, kh, fb (stock)
values increasing upwards: B, B/b, H/h, bs_1, fb (scion)
insignificant change: F/f, b, t, T/t
no change in the value: f, bs_2, bs, 1/2, H/kh

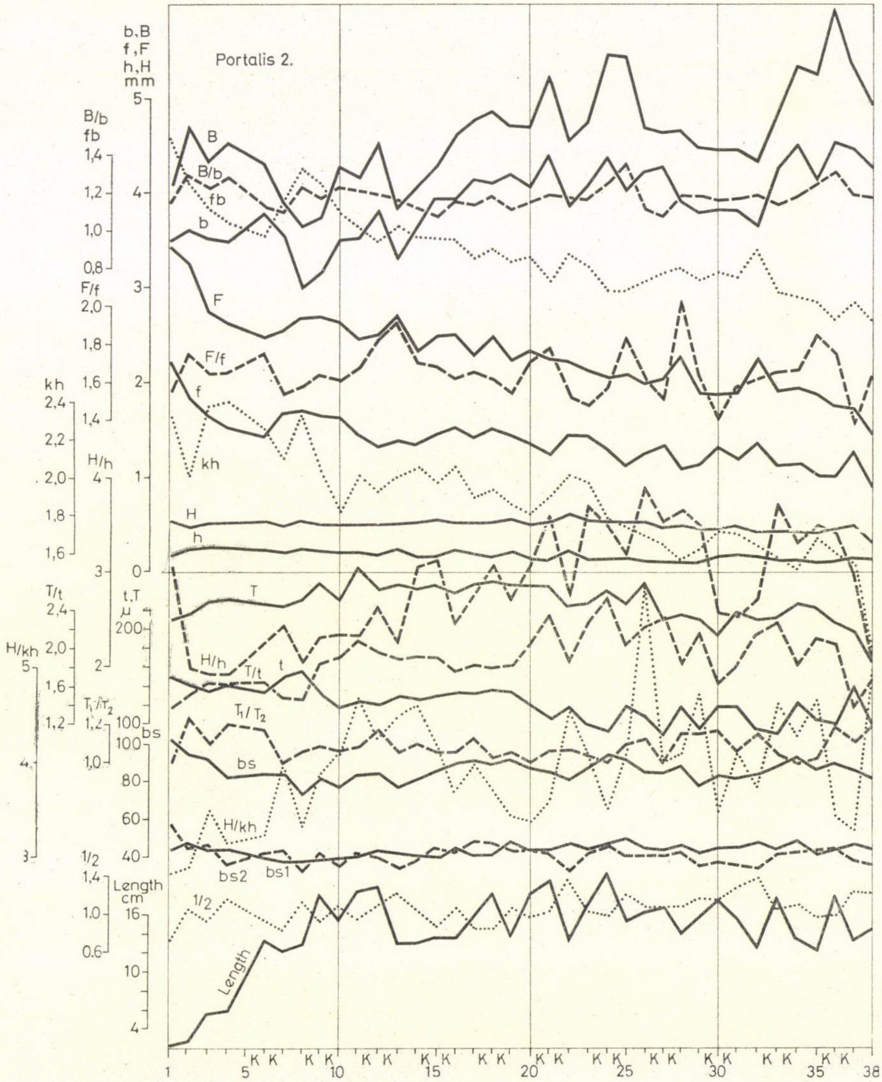

Fig. 1. Trends of the quantitative anatomical indices and of the length of internodes in the subsequent internodes. Portalis cane No. 2. On the abscisse the series of internodes (places of nodes with tendrils are indicated by K)

Table 1

Pith diameter

Section of cane				b	B	B/b
Olaszrizling		11—20	(1)	2381.12	2767.22	1.16
		21—30	(2)	2255.20	2737.04	1.21
Portalis	1	11—20	(3)	4049.36	4660.25	1.15
		21—30	(4)	3978.60	4739.35	1.19
	2	11—20	(5)	3863.08	4435.46	1.15
		21—30	(6)	4064.97	4811.16	1.18
	3	11—20	(7)	3571.68	4303.29	1.20
		21—30	(8)	3940.09	4799.71	1.22
5C	1	11—20	(9)	3326.08	3959.86	1.18
		21—30	(10)	3634.12	4465.64	1.23
	2	11—20	(11)	3727.79	4477.09	1.20
		21—30	(12)	3815.21	4829.89	1.26
	3	11—20	(13)	3154.36	3829.78	1.21
		21—30	(14)	3213.68	4154.47	1.29
F-test				53.76	31.92	4.44
		L.S.D$_{5\%}$		222.71	342.39	0.05

Discussion

The investigation results were analysed from two aspects: 1) whether there were consequent structural changes along the axis of the cane, and if so, of what character; 2) what was the relationship between our own results and the previous data concerning the differences between the various levels of canes.

The data presented show that in the first and last 6—8 internodes of the mature part of the cane considerable changes may occur, while in the intermediate 20—25 internodes the values remain more or less at the same level. A definite and expressed decreasing tendency was observed only in the case of the values of T. Definite decreasing or increasing tendencies characteristic of a certain variety were shown in the variety 5C by B/b and F, and in the variety Portails by h and H/h. This fact warns us to use these values with reservations when studying materials obtained from different levels. Nevertheless, the relatively small differences between the mean values and the rather low level of standard deviation suggest that the possibility of error is not very

Table 2

Xylem thickness

Cane section*	f	F	F/f	fb
(1)	1384.13	2039.77	1.48	1.336
(2)	1544.40	2098.05	1.36	1.463
(3)	1389.33	2240.63	1.62	0.844
(4)	1260.29	2098.05	1.67	0.775
(5)	1437.21	2441.48	1.70	0.945
(6)	1277.98	2081.40	1.64	0.762
(7)	1187.44	2190.67	1.88	0.879
(8)	1086.49	1817.06	1.68	0.670
(9)	1250.92	2144.88	1.75	0.956
(10)	1123.96	1989.82	1.80	0.773
(11)	1145.81	2010.63	1.88	0.776
(12)	1188.48	1824.35	1.56	0.709
(13)	945.99	1626.61	1.74	0.749
(14)	782.61	1388.29	1.83	0.598
F-test	12.13	24.92	13.47	42.64
L.S.D$_{5\%}$	294.52	148.82	0.11	0.105

* See in detail in Table 1.

high. It is especially the case when the samples have not been taken from definite levels but represent a mixed material.

When comparing the stock and vinifera canes we find that in the stock varieties the wood-pith ratio decreases while in the European variety increases upwards. Data of a single cane cannot be decisive, naturally, therefore we reviewed many unpublished records of ours and found the following: a wood-pith ratio decreasing upwards is generally characteristic of the root-stock varieties. Deviation from this rule was found only in the varieties Ganzin 1, 157 Pécs and du Lot, but even these varieties were not characterized by a wood-pith ratio improving upwards. Larger numbers of data are available only for a few of the European (*vinifera*) varieties. Of them the varieties Furmint, Hárslevelű, Leányka and Ezerjó are generally characterized by a wood-pith ratio improving upwards, while the Olaszrizling and Mézesfehér show varying tendencies. Our findings concerning the root-stock varieties correspond to those obtained by ZIMMERMANN (1952) in a three-level study as well as in a study according to internodes (ZIMMERMANN 1954), in which the proportion of wood decreases while that of the pith increases upwards in the cane. As for the European varieties only a single datum by ZIMMERMANN (1954) is available,

Table 3

Thickness and structure of phloem

Cane section*	h	H	H/h	kh	H/kh
(1)	213.0	606.0	2.87	2.77	3.79
(2)	225.0	597.0	2.67	2.74	3.58
(3)	193.5	552.0	2.89	1.93	4.42
(4)	150.0	517.5	3.49	1.82	5.17
(5)	198.0	540.0	2.76	1.96	4.05
(6)	156.0	519.0	3.38	1.76	4.20
(7)	201.0	571.5	2.88	2.03	3.74
(8)	129.0	486.0	3.85	1.53	4.66
(9)	174.0	601.5	3.52	2.14	4.95
(10)	154.5	597.0	4.12	2.16	7.97
(11)	165.0	595.5	3.69	2.07	4.90
(12)	174.0	573.0	3.36	1.95	4.64
(13)	141.0	510.0	3.67	1.96	5.39
(14)	127.5	471.0	3.94	1.70	6.03
F-test	15.89	15.14	8.07	37.81	4.23
$L.S.D_{5\%}$	21.6	33.0	0.47	0.16	0.94

* See Table 1. for the details

according to which the wood-pith ratio is highly fluctuating (the best is in the middle). Thus the statement that the European varieties — at least a part of them — are characterized by a wood-pith ratio improving upwards is new.

It is interesting to compare these results with investigations relating to the utilizability of various cane levels. Experiments with stock canes (COSMO 1948—50, NOVAK 1959, PASTENA 1960) unequivocally prove that the middle of the cane is the most suitable for propagation, followed by the base and finally the tip of the cane. Results obtained in studies on European (vinifera) canes are not so uniform as that. With the variety Ruhländer ZIMMERMANN (1954) found the following order: tip, middle, base; NOVAK's (1959) result with Riesling was: middle, base, tip. When carrying out experiments with the variety Merlot MIOTTO (1962) set up the following order: middle, tip, base. From this comparison the following conclusions can be drawn: the wood-pith ratio — though highly important — is not the only factor determining the usability of canes. The amount of nutrient reserves cannot cause the differences between the cane levels, as according to EIFERT *et al.* (1961) the 10th and 25th internodes of the root-stock cane do not considerably differ from each other in

Table 4

Trachea diameter

Cane section*	t	T	T/t	T_1/T_2
(1)	109.80	229.80	2.11	1.04
(2)	110.55	186.75	1.67	1.08
(3)	126.30	245.55	1.97	1.07
(4)	115.80	237.30	2.07	1.06
(5)	130.50	248.25	1.91	1.08
(6)	107.55	225.75	2.12	1.09
(7)	116.55	234.30	2.02	1.13
(8)	94.50	204.75	2.18	1.08
(9)	128.55	277.05	2.18	1.09
(10)	127.05	269.55	2.10	1.08
(11)	133.50	274.50	2.06	1.03
(12)	140.55	251.25	1.82	1.12
(13)	126.00	231.30	1.84	1.07
(14)	113.55	219.00	1.89	1.06
F-test	5.39	115.56	3.29	1.35
L.S.D$_{5\%}$	14.85	6.75	0.24	—

* See Table 1. for the details

sugar and starch content. The reason why the findings concerning the European canes are so divergent is that the varieties studied belong to different types: the Ruhländer and Merlot probably to a type with a wood-pith ratio improving upwards, while the Riesling to one where the wood-pith ratio does not improve upwards.

Changes in the inner structure along the axis of the cane have only been studied in some detail by ZIMMERMANN (1952, 1954). He examined the percentage proportions of pith, wood and living phloem in the surface of the cross-section, the minimum and maximum number of phloem fiber layers, the ratio between the minimum and maximum width of wood (histological value), the number of primary and total medullary rays, the ratio of pith and wood, as well as the dimension of the transporting surface. He performed the examinations partly in three levels (0.5—1 m, 2.0—2.5 m and 3.5—4 m), partly in each internode separately. His results concerning the number of medullary rays, the uneven thickness of the xylem as well as the number of phloem fiber layers more or less agree with our results, however, as to the diameters of wood and pith and the ratio between the two his findings correspond to our observa-

Table 5

Number of rays

Cane section*	bs_1	bs_2	bs	1/2	length of internode cm
(1)	40.8	28.2	69.0	1.45	11.40
(2)	41.9	30.5	72.4	1.39	9.15
(3)	43.6	41.7	85.3	1.05	16.06
(4)	43.1	38.2	81.3	1.16	15.14
(5)	43.3	42.9	86.2	1.02	15.72
(6)	45.7	40.5	86.2	1.14	16.39
(7)	41.0	38.0	79.0	1.08	14.20
(8)	45.8	36.0	81.8	1.28	16.09
(9)	47.1	29.4	76.5	1.64	16.58
(10)	48.7	26.2	74.9	1.97	16.76
(11)	48.3	31.4	79.7	1.61	17.33
(12)	49.5	31.8	81.3	1.61	17.42
(13)	42.7	20.0	62.7	2.34	16.51
(14)	47.2	15.6	62.8	5.16	16.35
F-test	12.53	25.8	23.40	5.30	8.73
$L.S.D_{5\%}$	2.3	4.4	4.5	1.29	2.22

* See Table 1. for the details

tions only for the stock varieties and not for the vinifera. We could not find any periodicalness occurring — according to Zimmermann — in the inner structure in relation with the position of the tendrils. It should be noted that even ZIMMERMANN (1954) made this statement only in the text, but it is not apparent at all in his graphs.

We can establish as a final conclusion that the increasing or decreasing tendency of the individual indices makes it necessary to choose a single definite cane level for quantitative anatomical comparisons. Since in the lower 6—8 internodes considerable changes may occur, for the purpose of examination the 10th internode is the most suitable.

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STUDIES ON THE RELATIONSHIP BETWEEN THE ZINC CONTENT IN THE KERNEL AND THE MATURITY GROUP OF MAIZE HYBRIDS

By

D. GYŐRI, M. PALKOVICS

DEPARTMENT OF SOIL SCIENCE, UNIVERSITY OF AGRICULTURAL SCIENCES, KESZTHELY

The zinc concentration in maize hybrids changes depending on their maturity group. In the later hybrids the zinc concentration decreased both in the endosperm and the germ + pericarp, with the exception of two hybrids of the 17 samples.

Introduction

There is only little information about the zinc content of the maize kernel in the literature. MASSEY—LOEFFEL (1966, 1967) dealt with the zinc content in the kernel of maize and determined the zinc content in the germ (+embryo), pericarp and endosperm (1967), in which the average levels were 138, 46, 9.9 ppm respectively. As beside these works we could not find any other publication in this subject, we decided to analyse the kernel components of the maize hybrids sufficiently widespread in Hungary.

We wanted to study the change in the zinc content of the maize kernel depending on the maturity group of the hybrids.

Material and Method

We divided the kernel components into two parts only (endosperm, germ(+ embryo) + pericarp) and analysed them for zinc content. After ashing the kernel components in a furnace (450 °C), the ash was dissolved in n HCl of 10 ml and the filtrate was evaporated to dryness. The dry sample residue was dissolved with an ammonia and ammonium chloride (1 M and 0.2 M) supporting electrolyte for the polarografic determination of zinc. The error of the method is lower than ±10 per cent. In this paper we studied 6 hybrids (17 samples belonging to different maturity groups). The samples were obtained from the state farm of Bábolna in Hungary.

Results

In Table 1 we can see the average distribution of the zinc concentration of the maize kernel.

These results indicate that the germ(+ embryo) has the highest zinc concentration and zinc content too. The second in order is the concentration of the pericarp. The endosperm has the lowest zinc concentration.

Our results coincide with the data of Massey concerning the principal tendency of zinc distribution in the kernel components. The zinc analytical data to be found in Table 2 give information about the zinc concentration in the kernel components depending on the maturity group of the hybrids. Between the two fractions of the maize kernel there are important differences. According to the data of MASSEY—LOEFFEL (1967) a correlation between zinc concentration of kernel and kernel parameters for 31 inbred lines was found only in one case, namely between zinc concentration in the pericarp and zinc

Table 1

Average distribution of zinc concentration of maize kernels

Fractions	Zn in one grain	Zn ppm	% of total zinc	% of total weight
Endosperm	2.28	9.0	18.9	55.4
Pericarp	4.16	30.5	34.4	31.8
Germ	5.64	96.5	46.7	12.8

Table 2

Zinc concentration in kernel components of maize

Maize hybrids	Zn ppm		
	endosperm	germ (+embryo)+pericarp	
MVSC 202	9.0 ±0.5	30.5	±1.0
MVSC 458	7.0 ±0.5	21.0	±0.5
MVSC 680	6.0 ±0.5	14.0	±0.5
MVTC 201	8.5 ±1.0	33.7	±1.2
MVTC 414	6.0 ±0.5	23.2	±0.7
MVTC 651	3.5 ±0	21.5	±0
OSSK 212	10.5 ±1.0	28.7	±4.2
OSSK 366	6.0 ±0.5	16.2	±1.2
OSSK 619/1	7.0 ±0.5	32.5	±1.5
SzSC 300	9.5 ±1.0	40.0	±0.5
SzSC 350	5.0 ±0.5	26.0	±0.5
SzSC 400	8.7 ±1.2	22.5	±1.0
SzSC 460	8.0 ±0.5	28.7	±1.2
KDC 341	7.5 ±0	36.2	±1.2
KDC 593	5.0 ±0.5	27.0	±1.5
GTC 339	5.0 ±0.5	27.0	±0.5
GTC 488	5.0 ±0.5	20.5	±1.0

concentration in the whole kernels. Besides they stated that the highest zinc concentration was in the germ (+ embryo) and it was lower in the pericarp and lowest in the endosperm. At the same time it was a very important fact, that the endosperm had the highest weight percentage (about 80%) among the kernel components.

In possession of these results we decided to analyse only two fractions of the kernel components, because in our opinion this is also a way of getting appropriate information about the typical distribution of the zinc content in the kernel of different maize hybrids.

Our data (Table 2) indicate that the endosperm has a lower zinc content, than the inside fractions of the kernel (germ + embryo + pericarp).

The zinc concentration is three-five times more in the inside fractions than in the endosperm. Among the examined hybrids the zinc concentration is lower in those belonging to later maturity groups except the behaviour of SzSC 460 Hungarian hybrid, which departs from this rule. In our opinion this rule is very important, because in later maturity groups the zinc concentration may decrease due to two causes: 1) The plants are no longer in need of zinc in their later growing phase, 2) The plants cannot take up the required zinc quantity because of the weather or soil conditions.

The first reason allows us to suppose that hybrids belonging to late maturity groups do not require higher zinc concentration for their growth, or referring to the second reason, the plants would require higher zinc concentration, but they cannot take it up because of some hindrance which may be external circumstances (for example, soil condition) or internal ones (genetic property).

The low zinc concentration of the hybrids belonging to late maturity groups may be in connection with the synthesis of proteins or some amino acids. Later we would like to investigate this problem.

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EFFECT OF FERTILIZER RATES AND IRRIGATION ON FUSARIUM INFECTION OF WINTER WHEAT

By

E. Bocz, El-S. Hefni, O. Tóth

DEPARTMENT OF AGRONOMY, UNIVERSITY OF AGRICULTURAL SCIENCES, DEBRECEN;
DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE,
AIN SHAMS UNIVERSITY, CAIRO

The *Fusarium* diseases of wheat have received the attention of investigations throughout the world for decades. In this investigation the kernels shrivelled, the 1000-kernel weight and germination per cent decreased due to *Fusarium* diseases when the doses of nitrogen fertilizer were high. The highest mean per cent of germination in unirrigated subplots was 88 per cent while the lowest mean was 82.5 per cent obtained at the highest rates of fertilizer. In the irrigated subplot, the highest mean of germination was 90 per cent obtained from the control while the lowest mean was 81 per cent obtained from the highest rates of fertilizer. The baking quality gradually improved by increasing the doses of nitrogen fertilizer up to 300 N kg/ha, without irrigation, while it reached its peak at 200 N + 100 P_2O_5 + 100 K_2O kg/ha of fertilizer rates with irrigation.

Introduction

Tóth (1970) reported that an epidemic *Fusarium* infection on cereals effected the quantity and quality of crops in Hungary, and the yield decreased 7—8.7 q/ha but Kükedi (1971) found in some plots that it remained below 17.4 q/ha. Lelley (1965) reported the *Fusarium* diseases of wheat as early as in the year 1965 which he observed in the Great Hungarian Plain.

According to Ubrízsy (1965) and Schmiedeknecht (1969) the infection of *Fusarium* was affected by moist weather and large N-doses. In the opinion of McKnight—Hart (1966) and Domsch *et al.* (1969) the diseases due to the *Fusarium* pathogens are increased on wheat by systematic irrigation.

Szepessy (1967) explained that the nitrogen demand of wheat can be satisfied even to the maximal degree — in different quantities according to the species — but exaggerated N-abundancy must be counterbalanced by P- and K-fertilizers at the necessary rate. Tóth—Dely (1968) and Dankó—Tóth (1969) proved that *Fusarium* diseases had increased in Hungary and caused many troubles in the maize crop. Bockmann (1968) reported that delayed ripening increases the infection of *Fusarium culmorum*, the chaff leaves of the spikelets of the infected kernels became white, kernels having *Fusarium* were shrivelled and withered, the 1000-kernel weight and percent of germination decreased. Malalasekera—Colhoun (1968) found that the germination percentage should perhaps be considered since it was noted that in soil uninoculated by

Fusarium culmorum, the time between sowing and seedling emergence above the soil was shorter in the case of seeds soaked for 3 h than in any other of the treatments.

SNYDER—NASH (1968) reported that applying nitrogen fertilizer to the soil increased the severity of the disease. COLHOUN *et al.* (1968) emphasized that the influence of soil reaction and depth of sowing on the incidence of the disease caused by *Fusarium* spp. is not as substantial as are the effects of spore load, soil moisture or temperature. MILLAR—COLHOUN (1969) found on wheat that samples having 30 per cent of the seeds infected with *F. nivale* gave 97—100 per cent germination, without surface sterilization, on malt agar at 13—14 °C. KÜKEDI (1971) mentioned on winter wheat that by adding 40, 80 and 160 kg/ha of nitrogen fertilizer without K_2O, the *Fusarium* increased by 17 per cent, 23 per cent and 38 per cent, respectively. He also found that the 1000-kernel weight in plots which were attacked by fusarium was 22.2 g, while that of the normal kernels were 45.1 g and the number of kernels per spike decreased due to *Fusarium* disease in the higher fertilizer rates in the case of the Besostaya wheat variety. He also showed that the grain yield of winter wheat in Hungary in 1970 was 17.4 q/ha in the regions, attacked by fusarium, while in the same year the average grain yield of the country was 21.4 q/ha according to the agricultural statistics of Hungary. In most cases not only the 1000-kernel weight decreased, but the number of kernels per spike and the weight of kernels per spike also decreased.

Apart from the factors effecting reduction of crop and decay of quality due to *Fusarium* infection such as monoculture, previous plants, species of plant, infected grains of sowing, density of plants, types of soil, climatic circumstances, the rates of fertilizer applied and proportion of NPK play an important role besides the quantity of inoculators accumulated in the soil and the organic contents of the soil.

Mrs. ORSZÁGH (1971) dealt with the present tasks of the fight against the diseases of wheat using the newest data and her own results of research, among others the factors effecting diseases of wheat are analysed referring to the fertilizer and irrigation.

Material and Method

This investigation was carried out in a field trial at Nagyhegyes irrigation farm, Debrecen, Hungary in 1969/70. A winter wheat variety was used, namely Besostaya (*T. aestivum*). The sowing time was October 1, 1969 and the harvest time was July 18, 1970. A split-plot design with four replications was used.

The levels of irrigation treatment were in the main plot, while the rates of fertilizer the subplots. The treatments used for irrigated and non-irrigated main plots were: 1. 0-rate as a control, 2. 100 N + 100 P_2O_5 + 100 K_2O kg/ha. 3. 200 N + 100 P_2O_5 + 100 K_2O kg/ha. 4. 300 N + 100 P_2O_5 + 100 K_2O kg/ha. The water was added at the heading stage. The fertilizer rates were added once before sowing. The nitrogen fertilizer was applied in the form of ammonium nitrate (34 percent N), phosphorus in the form of superphosphate (18 per cent P_2O_5)

and potassium in the form of potassium chloride (40 per cent K_2O). Samples of seeds were collected from the subplots, and data were taken from the average of four subplots, and data were taken from the average of four replications on: 1) the percent of seeds, attacked by *Fusarium* and other Fungi outside and inside, which did not germinate or died after germination. 2) The germination per cent of seeds attacked by *Fusarium* and other fungi outside and inside and did not die after germination. 3) The quality of seeds. 4) The protein content of grain.

The fusarium test was performed on the seeds by using Papavisas method (cited by MALALASEKERA—COLHOUN, 1969).

The crude protein was obtained by using Kjeldahl's method. The moisture of the gluten was obtained by using the undistilled water method, the Hungarian standard method (MSZ 6369/5—70) was used in gluten plasticity. The baking quality was obtained by multiplying the percentage of moist gluten and the relative number of plasticity.

Results

On the surface of the kernels obtained from the experiment with fertilizers the *Fusarium* infection could be found scarcely in all cases, but the presence of conidia sticking on the seed-coat or mycelia in the seed-coat, did not cause a qualitative damage that would change the values of the inner contents decisively. Per cent of germination and the values of the inner contents were affected by fungi settled within the seed-cover and living there.

In the present experiment *Fusarium* infection as described above was high related to the average of the country, but it effected the results of crop, the percentage of germination after all. It decreased the 1000-kernels weight and influenced the values of inner contents related to the earlier years. The loss was moderated partly by the fact, that the *Fusarium* infection took place before harvesting time at the time of vegetation, on the other hand the rate of applied K_2O and P_2O_5 fertilizer had a favourable effect even when the rate of Nitrogen fertilizer was increased.

The decrease in the germination percentage, parallel to the deep infection of kernels, showed a scarcely consequent positive correlation with the increase of the N-rate both in irrigated and non-irrigated treatments, and the germination percentage was always lower than that of kernels obtained from the control plots. The germination percentage was 5.5 per cent lower than that obtained by adding 300 N kg/ha without irrigation, 9 per cent lower by applying irrigation related to the control.

Though the change in the values of the inner contents did not show any strict consequency in the various treatments, the higher N-rate could not assert itself positively in the crude protein content and in the moist gluten content as it did in the years free from *Fusarium*. The baking quality did not improve significantly either in the examined samples. The presence of *Fusarium roseum* v. *graminearum*, *Fusarium culmorum*, and *Fusarium oxysporum* could be proved. Besides these three *Fusarium* species more saprophytic fungi could be isolated from the kernels: the species of *Penicillium*, *Alternaria*, *Aspergillus*, *Rhizopus*, *Mucor*, *Helminthosporium*, and *Trichothecium* were found most frequently.

Table 1

Rate and effect of Fusarium and other fungi on germination of wheat by applying N fertilizer rates

Treatments	Without germination								Germinated, died	Without germination (*died*)				Germination %
	1	2	3	4	1	2	3	4		1	2	3	4	
a) *non-irrigated:*														
unfertilized (control)	3	3	1	2	—	3	—	—	12	—	59	—	29	*88*
N/100+ P_2O_5/100+K_2O/100	7	3	3	1	2	1	—	—	17	—	48	—	35	83
N/200+ P_2O_5/100+K_2O/100	5	1	—	2	2	4	1	4	19	—	42	2	37	81
N/300+ P_2O_5/100+K_2O/100	8	—	—	2.5	—	5	—	2	17.5	—	40.5	3	39	82.5
b) *irrigated:*														
unfertilized (control)	6	—	—	2	—	2	—	—	10	—	57	—	33	90
N/100+ P_2O_5/100+K_2O/100	6	3	1	3	—	2	—	2	18	—	35	—	47	82
N/200+ P_2O_5/100+K_2O/100	7	—	1.5	6	—	2	—	—	16.5	—	34	1	48.5	83.5
N/300+ P_2O_5/100+K_2O/100	9	1	—	1	2	5	—	1	19	—	42	—	39	81

1 = infected with *Fusarium* (heavy infection with lesion of germ)
2 = infected with *Fusarium* (on the surface)
3 = infected with other fungi
4 = infected with fusarium and other fungi

Table 2

Change of values of inner contents affected by N-fertilizer given and increasing doses besides Fusarium *infection*

Treatments	crude protein %	moist gluten %	plasticity %	baking quality
a) *non irrigated:*				
unfertilized (control)	14.92	40.14	9.5	55.07
N/100+P_2O_5/100+K_2O/100	13.98	40.00	9.0	56.6
N/200+P_2O_5/100+K_2O/100	15.36	40.70	6.0	65.52
N/300+P_2O_5/100+K_2O/100	15.54	41.1	6.0	66.17
d) *irrigated:*				
unfertilized (control)	14.48	38.15	5.5	67.43
N/100+P_2O_5/100+K_2O/100	15.18	40.35	5.0	67.58
N/200+P_2O_5/100+K_2O/100	15.5	43.75	6.0	70.43
N/300+P_2O_5/100+K_2O/100	15.14	42.3	6.0	68.1

The worse yield represents a more complicated problem from the point of view of pathology that can be explained more exactly only by a complex examination before harvest and by its analysis. The spring with much precipitation following the green winter and the rainy summer promoted not only the *Fusarium* infection, but also the development of powdery mildew (*Erysiphe graminis*), in the monoculture and in low-lying places *Ophiobolus graminis* and *Cercosporella herpotrichoides* were also observed.

An examination of many and more infected samples would promote a better knowledge of these complicated connections that can also be seen from the tendency of the examinations described above.

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EFFECTS OF METHODS OF EMASCULATION AND ISOLATION, TIME OF POLLINATION AND ORIGIN OF POLLEN ON FRUIT SETTING IN PEAR FLOWERS

By

J. NYÉKI

RESEARCH INSTITUTE OF HORTICULTURE, BUDAPEST

Effects of methods of emasculation and isolation, time of pollination and origin of pollen on fruit setting in pear flowers were studied between 1968 and 1970. Radical emasculation was just as efficient as the removal of anthers. It is much more feasible, rapid and reliable in practice than the removal of anthers. Of the methods of isolation, the application of parchment paper bags proved the best, in the cellophane bags the emasculated flowers dried. Isolation in net gabs was not suitable for excluding the possibility of cross-pollination. The optimum time of pollination varied according to the stage of flowering and variety. The highest percentage fruit setting was obtained with pollination carried out in full blossom (stigma covered by shiny secretion) and at the stage of bud bursting. No significant differences in the fertilizing ability and the extent of pollen tube formation were found between the collected pollen and that obtained directly from the isolator.

Introduction

Pear varieties grown in Hungary are completely autosterile, while certain varieties are autofertile to different extents (NAGY 1960, MALIGA 1961, BRÓZIK—NYÉKI 1970). Natural parthenocarpy is a frequent phenomenon in pear varieties to which varieties are disposed in different degrees (NYÉKI 1973). When studying the conditions of fertilization the authors used different methods of emasculation and isolation (cf. BRÓZIK—NYÉKI 1970). The need for elucidating the fertilization conditions of pears (selfing, parthenocarpy, cross-pollination) required further methodological studies.

The following questions had to be answered: 1. Is there any difference in natural parthenocarpic fruit developing ability between pear flowers radically emasculated and those whose anthers are removed? 2. What is the influence on the extent of fruit setting of various materials (parchment paper bag, cellophane bag, net bag) applied for isolation? 3. In which stage of flowering does pollination give the highest percentage of fruit setting? 4. Is there any difference in fertilizing capacity between the collected pollen and that originating directly from the isolator?

Table 1

Effect of the method of emasculation on fruit setting in pear flowers. Means of isolation: parchment paper. Time of emasculation: bud bursting
(1968—1970)

Variety	Year	Method of emasculation				Without emasculation (self pollination)	
		Radical		Removal of anthers			
		Number of treated flowers	Percentage of ripe fruits	Number of treated flowers	Percentage of ripe fruits	Number of flowers	Percentage of ripe fruits: full seed per fruit
Arabitka (G)	1968	736	21.9	527	23.4	935	31.4 : 0.0
	1969	572	38.5	611	41.3	872	39.5 : 0.0
	1970	628	23.2	734	25.7	981	24.3 : 0.0
Pringalle vaj	1968	1015	0.0	974	0.0	627	1.7 : 0.0
	1969	989	12.0	875	11.7	811	13.2 : 0.0
	1970	737	0.6	812	1.1	803	1.3 : 0.0
Vilmos körte	1968	1115	0.0	1223	0.0	1102	0.0 : 0.0
	1969	859	0.0	790	0.0	1018	0.0 : 0.0
	1970	1278	1.0	1400	1.5	975	0.0 : 0.0
Hardenpont téli vaj	1968	728	1.8	812	2.3	852	0.0 : 0.0
	1969	971	4.2	835	4.8	937	0.0 : 0.0
	1970	857	1.9	812	2.1	1049	1.1 : 0.0

Material and Method

The investigations were carried out between 1968 and 1970 in the Érd-Elvira Station of the Research Institute of Horticulture with trees of a variety collection grafted to wild pear stocks planted in 1953. For methodological reasons all flowers of the cymes were used in the study irrespective of their different stages of development and nutrition conditions, namely Daniel (1962) pointed out considerable differences in the extent of fruit setting as a result of flowers removed from the cyme.

Treatments were applied in the middle zone of the crown, at all cardinal points, in more than one trees of each variety in order to have a great number of flowers for the evaluation of results.

Results

Effect of the method of emasculation on fruit setting. Some authors consider radical emasculation, while others the removal of anthers as suitable for emasculating various fruit species. Mándy (1964) summarized the methods suggested for the different species. Okályi—Maliga (1956) found the best method to be the removal of anthers for pomiferous fruits and radical emasculation for stone fruits. When crossing berry fruits Szilágyi (1961), Seljahudin

Non-emasculated flowers (self pollination)							
Method of isolation							
Net bag		Parchment paper		Cellophane		Net bag	
Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits
859	29.8	935	31.4	835	20.3	1112	48.5
776	37.5	872	39.5	712	19.5	850	52.7
921	26.4	981	24.3	677	18.3	921	39.8
715	5.1	627	1.7	598	0.7	737	3.7
823	21.2	811	13.2	879	3.2	497	15.6
619	4.9	803	1.3	1157	0.0	681	4.9
537	1.7	1102	0.0	973	0.0	839	2.8
945	2.8	1018	0.0	827	0.0	671	1.7
1357	3.3	975	0.0	889	0.0	819	4.6
1211	3.5	852	0.0	1279	0.0	1117	5.8
857	6.9	937	0.0	1100	0.0	1220	7.3
938	4.7	1049	1.1	1018	0.0	675	8.7

—Brózik (1965) and Tóth *et al.* (1966) used the method of radical emasculation of fully developed flower buds.

In our experiments the effect of radical emasculation and removal of anthers on fruit setting was studied in four pear varieties differently inclined to parthenocarpy (Nyéki 1974) (Table 1). According to the results of the investigations no significant difference in fruit setting was found between flowers subjected to radical emasculation and those only deprived of their anthers.

Effect of the method of isolation on fruit setting. The methods suitable for excluding the possibility of cross-pollination were found to vary from species to species (Mándy 1964). In our experiments the effects of three isolation methods (parchment paper-, cellophane- and net bags) on fruit setting were studied in four pear varieties (Table 2). From each type isolators of the size of 25 ×35 cm were used for the isolation of the flowers. Each isolator contained 25—30 flowers. As shown in the tables, the highest percentage of fruit setting was obtained in flowers isolated with net bags, while the lowest in those isolated with cellophane. Emasculated flowers isolated with cellophane were found to dry up in a short time.

Table 2

Fruit setting response of pear flowers to the method of isolation. Time of emasculation: bud bursting (1968—1970)

Variety	Year	Radically emasculated flowers			
		Method of isolation			
		Parchment paper		Cellophane	
		Number of treated flowers	Percentage of ripe fruits	Number of treated flowers	Percentage of ripe fruits
1. Arabitka (G)	1968	736	21.9	637	14.1
	1969	572	38.5	852	18.6
	1970	628	23.2	721	10.2
2. Pringalle	1968	1015	0.0	583	0.0
	1969	989	12.0	749	2.5
	1970	737	0.6	829	0.0
3. Vilmos körte	1968	1115	0.0	935	0.0
	1969	859	0.0	1012	0.0
	1970	1278	1.0	1129	0.0
4. Hardenpont téli vaj	1968	728	1.8	939	0.0
	1969	971	4.2	857	1.1
	1970	857	1.9	742	0.0

As to the seed content of fruits, those developed from flowers isolated with parchment paper or cellophane were all found to be parthenocarpic, while fruits developed from flowers isolated with net bags contained 1—3 germinative seeds on an average. The results agree with those obtained by others. E.g. TÓTH—TÓTH (1959) pointed out that with net bags used as isolators fruit setting was almost 10 per cent in the emasculated gooseberry flowers compared to the 0.2 per cent of those isolated with cellophane, while fruit setting was similar in non-emasculated flowers.

Effect of pollination performed at different stages of flowering on fruit setting. In four pear varieties VISSER (1955) found the highest percentage of fruit setting when pollination was carried out at the red bud stage; both earlier and later pollinations gave worse results. In stone fruits too, pollination performed at an early stage of flowering yielded the highest percentage of fruit setting (BRADBURY 1929, TUKEY 1933, STÖSSER 1966).

Investigations were performed on two pear varieties, at three flowering stages (bud bursting, full blossom, beginning of flower shedding). The variety Hardenpont téli vaj was used as pollen donor and proved compatible with

Table 3

Fruit setting trends in flowers pollinated at different stages of flowering
(1968—1969)
(Pollination: with pollen originating from flowers held brush-like together.
Isolation: Parchment paper)

Variety ♀	Stage of flowering	1968				1969			
		Free pollination		Pollinated with pollen from Hardenpont ♂		Free pollination		Pollinated with pollen from Hardenpont ♂	
		Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits	Number of treated flowers	Perc. of ripe fruits
1. Clapp kedveltje	Bud bursting			872	3.7			921	12.5
	Full blossom (stigma covered with shiny secretion)	1350	1.9	758	4.2	1120	18.3	1012	21.3
	Beginning of flower shedding			887	0.5			975	2.1
2. Vilmos körte	Bud bursting			914	5.4			769	13.8
	Full blossom (stigma covered with shiny secretion)	1172	4.2	831	3.5	1025	5.4	847	10.3
	Beginning of flower shedding			1227	1.4			918	2.6

both varieties as to fruit and seed setting (Brózik—Nyéki 1970). The results showed (Table 3) that the highest fruit setting percentages were obtained when pollination was performed either at the stage of bud bursting (Vilmos körte) or in full blossom (stigma shiny, covered with secretion — Clapp kedveltje).

Our results agree with those obtained by Eaton (1959, 1962) in cherry varieties. Eaton found that on the second day after bud bursting the number of active egg-cells rapidly decreased. Four days after opening 80 per cent of the egg-cells were degenerated and on the sixth day no active egg-cell could be found.

Fertilizing ability of pollen collected in different ways. In our experiments the fertilizing ability of pollen collected according to the method described by Barrett—Ariaumi (1952) and King (1955) on one hand, and of that taken immediately before pollination from flowers held together brush-like in isolators, on the other (Table 4), was investigated. According to the results, pollination carried out with the collected pollen gave lower fruit setting percentage than when performed with pollen originating directly from the isolator. Differences between the two treatments were, however, not significant. There is no significant difference between the two ways of obtaining pollen in the extent and dynamics of pollen tube formation either (Nyéki, non-published).

Table 4

Fertilizing ability of pollen collected in different ways
(1968—1969)
(Pollination: in full blossom — stigma covered with shiny secretion.
Isolation: parchment paper bag)

Variety ♀	Year	Pollen variety ♂	Fertilizing ability of pollen			
			collected pollen		pollen originating from flowers held brush-like together	
			Number of treated flowers	Percentage of ripe fruits	Number of treated flowers	Percentage of ripe fruits
1. Clapp kedveltje	1968	Hardenpont	647	3.5	758	4.2
	1969	téli vaj	928	17.5	1012	21.3
2. Vilmos körte	1968	Hardenpont	712	2.7	831	3.5
	1969	téli vaj	957	5.2	847	10.3

Conclusions

In fruit varieties radical emasculation can be successfully used for fertilization and breeding studies; it does not influence the extent of fruit setting. Radical emasculation is much more feasible, rapid and reliable in practice than the removal of anthers.

Among the methods of isolation (parchment paper, cellophane and net bags) parchment paper bags are the most suitable both for excluding the possibility of cross-pollination and for ensuring optimum fruit setting. Net bags render cross-pollination possible and are not, therefore, suitable for isolation purposes. Flowers isolated by cellophane show rapid senescence and drying up in the isolators.

The time of pollination proved to be one of the most important factors determining the extent of fruit setting. The optimum time of pollination differs from variety to variety. Pollination performed in full blossom (when the stigma is covered with shiny secretion) and before gave the highest fruit setting percentages. When pollination was carried out later the fruit setting values obtained were not suitable for characterizing the compatibility conditions of the varieties.

There are no significant differences in fertilizing ability and the extent and dynamics of pollen tube development between the pollen collected and that obtained directly from the isolator. Pollens obtained in either way are suitable for studying compatibility in the varieties.

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THE EFFECTS OF MALEIC HYDRAZIDE ON HALF-RIPE APRICOTS AND PLUMS, AND ITS REVERSION WITH ASCORBIC ACID

By

D. SURÁNYI

HORTICULTURAL RESEARCH STATION, CEGLÉD

The effects of maleic hydrazide, ascorbic acid, and the joint effect of these compounds on half-ripe fruits of the apricot varieties C.235 "Magyar kajszi" and C.778 "Rózsabarack" and of the plum variety "Besztercei szilva" were studied. The lowest loss of storage was obtained with a 2000 ppm maleic hydrazide treatment, but the concentration of 500 ppm was also found satisfactory. Ascorbic acid even at a concentration of 1000 ppm was able to stimulate the weight loss of fruits to a great extent. The results of the experiments show a positive correlation between the dry matter content and the loss of storage and a negative one between the ratio of invert sugar to acid and the loss of storage. Least damage was done to fruits with a high ratio of invert sugar per acid. As a result of a combined treatment at 500 ppm synergism could be demonstrated, which regarding its effectivity was equivalent to the strongest effects of ascorbic acid. From this fact the author has drawn the conclusion that the efficiency of maleic hydrazide treatments in the course of spraying is a function of the bio-level of ascorbic acid in the fruit.

Introduction

Maleic hydrazide was first synthetized in 1895 (Curtius — Foersterling cit. BRUCKNER 1964). Its effects on the physiology of plants were first reported by SCHOENE—HOFFMAN (1949). As a result of its numerous favourable effects it is widely used to inhibit germination in root and tuberous plants, retain the growth of laterals in tobacco, induce male sterility (e.g. in maize); it is, further, an important herbicide too (ZUKEL 1957).

Maleic hydrazide is generally known as a compound of anti-auxin character (LEOPOLD—KLEIN 1951, PILET 1961). Beyond this a number of publications give account of the fact that of the nucleic acid bases it is antagonistic to uracyl and cytosine and inhibits their incorporation into the nucleic acid fractions (RAKITIN *et al.* 1971). The inhibiting effect of maleic hydrazide on protein synthesis which results in a slowing down of the development processes (germination) is connected with this.

In our investigations we studied the question of whether maleic hydrazide is able to slow down the ripening process of half-ripe *Prunus* fruits on one hand, and whether on the other hand, another compound — ascorbic acid — which exercises the opposite influence is able to eliminate the effects of the former, since this is supposed to be one of the chemical factors determining the efficiency of maleic hydrazide treatments.

Material and Method

Half-ripe fruits of the apricot varieties C.235 "Magyar kajszi" (n = 9) and C.778 "Rózsabarack" (n = 10) and of the plum variety "Besztercei" (n = 10) were treated with ascorbic acid and maleic hydrazide of 500, 1000, 2000 and 4000 ppm concentration and with the combination of the two chemicals. Fruits were stored at +4 °C for three weeks after the treatment. Due to the different ripening times of the varieties treatments were applied to "Magyar kajszi" on 17th July, to "Rózsabarack" on 31st July and to "Besztercei" plum on 4th September. The fruits were injected with 0.5 cm^3 solution and tap water (for control), that is, differences in size between the fruits meant different quantities of active agent per unit fruit weight. Thus the treatment of 4000 ppm represented 0.05 and 0.10 ppm active agent with the apricot varieties "Magyar kajszi" and "Rózsabarack", respectively, and 0.15 ppm active agent in the case of the plum variety "Besztercei" per 1 g pure flesh. Lower concentrations mean, of course, less active agent, in accordance with the rate of dilution. In the case of a combined treatment half of the 40.5 cm^3 was an appropriate concentration of ascorbic acid, while 0.25 cm^3 was that of maleic hydrazide.

Weight loss during storage as a percentage of the initial fruit weight was also a subject of investigations, and at the end of the storage dry matter, invert sugar and acid contents were determined. The two latter were reduced on the basis of the calculated ratio of invert sugar per acid. The results were evaluated by means of variance analyses, and correlation calculations were also made to determine the relationship between storage losses and the component indices studied.

The effects of ascorbic acid, maleic hydrazide and combined treatments were evaluated marking the treatment representing the lowest numerical value with No. 1., etc. in all three cases (storage loss, dry matter content, invert sugar/acid ratio). Thus, when summarized, the numbers of placement given by the three varieties for storage loss, dry matter content and ratio of invert sugar/acid were minimum 3×1 and maximum 3×13. It was in this way that we calculated the correlation value (r) by which we wanted to prove that no treatment effect appeared by itself; a given loss of storage was the result of simultaneous changes in more than one parameter.

Results

Ascorbic acid generally increased the storage loss of "Magyar kajszi" apricots, which was even more remarkable after the combined treatments. Maleic hydrazide did not moderate the weight decrease; at lower concentrations it even surpassed the control. As to the dry matter content, differences occurred only between the individual treatments of which the ascorbic acid treatment at a concentration of 500 ppm and the 2000 ppm combined treatment were the most outstanding ones. The ratio of invert sugar/acid shifted toward the invert sugar to the highest extent under the influence of ascorbic acid, though the results of combined treatments were not much different either (Table 1).

The apricot variety C. 778 "Rózsabarack" gave a different response to the treatments compared to "Magyar kajszi". With a single exception all treatments were able to reduce weight loss during storage. The chemicals had a favourable effect on the dry matter content, but not on the quantity of invert sugar, which is not discussed in detail in the present paper. On the other hand, the inhibition of acid decomposition as a consequence of the treatments was more intensive, so — after all — the ratio of invert sugar/acid did not differ from the results obtained for the apricot variety C. 235 "Magyar kajszi".

Table 1

Effects of ascorbic acid (AA) maleic hydrazide (MH) and of the combination of these two compounds on half-ripe fruits of the apricot varieties C.235 "Magyar kajszi" (MK) and C.778 "Rózsabarack" (RB) and of the plum variety "Besztercei" (Bsz)

Concentration ppm		Weight loss from storage weight %			Dry matter %			Ratio of invert sugar/acid		
		MK C. 235	RB C. 778	Bsz	MK C. 235	RB C. 778	Bsz	MK C. 235	RB C. 778	Bsz
Control (tap water)		2.99	26.57	6.40	13.0	16.0	20.3	6.60	9.40	31.12
4000	AA	5.25	20.09	9.21	14.4	16.8	20.9	7.25	9.32	27.88
	MH	2.92	19.50	6.88	14.7	16.0	20.5	7.11	10.32	29.51
	AA+MH	6.43	16.06	6.14	14.4	16.2	21.0	6.28	9.64	30.25
2000	AA	3.61	16.68	7.02	13.3	16.5	20.2	6.68	9.66	27.24
	MH	2.92	17.07	6.17	14.4	15.1	19.7	7.13	10.57	30.30
	AA+MH	4.73	12.72	5.74	14.8	16.1	19.6	7.59	7.74	31.41
1000	AA	4.46	17.11	7.33	14.3	16.0	21.1	7.27	8.58	26.46
	MH	3.74	18.61	6.43	13.3	15.5	20.0	6.93	9.18	26.10
	AA+MH	5.53	12.04	6.81	14.3	15.3	18.8	7.14	8.40	33.72
500	AA	3.64	23.42	7.30	14.7	16.3	21.0	7.88	8.93	28.51
	MH	3.30	17.09	6.50	14.0	16.3	19.6	7.11	9.36	31.12
	AA+MH	8.54	13.81	8.26	14.2	16.6	22.8	6.32	8.95	28.50
L.S.D. 5% s.d.$_{5\%}$		1.52	3.05	1.48	0.09	0.09	0.17	0.51	0.92	2.60
L.S.D. 1% s.d.$_{1\%}$		2.02	4.05	1.97	0.12	0.12	0.24	0.67	1.22	3.51
L.S.D. 0.1% s.d.$_{0,1\%}$		2.61	5.22	2.54	0.17	0.17	0.34	0.89	1.63	4.70

When studying the weight loss of the plum variety "Besztercei" during storage we found that the 500 ppm combined treatment had the same, or nearly the same effect as the ascorbic acid treatments. This can be considered an established fact in the case of the apricot varieties "Magyar kajszi" and "Rózsabarack" too (Table 2).

All this is well demonstrated in Table 2 as well; in the succession of storage loss, dry matter content and invert sugar/acid ratio the following values were obtained after 4000 ppm ascorbic acid and 500 ppm combined treatments: 30 and 28.5, 32 and 32.5, 21 and 23, respectively. Dry matter content increased mainly as a result of ascorbic acid treatments, while the ratio of invert sugar/acid could be raised only by the 1000 ppm combined treatment.

Before summarizing our results obtained for the three varieties we present here the results of correlation calculations made with the basic data used. According to Table 3, the most reliable correlations are found between dry matter content and storage loss as well as between invert sugar/acid ratio and storage loss. In treatments giving a higher ratio of invert sugar/acid the original weight of some fruits was reduced to a lower extent.

The above findings are also confirmed by Table 2. By arranging the results of the treatments in an order of succession we succeeded in giving a clear picture of the differences between the individual treatments as well as between the concentrations. But even when summed up, as results of grade correlations they are convincing as to correlations between dry matter content and storage loss, and between invert sugar/acid ratio and storage loss (Table 3).

The results of maleic hydrazide injected into plums confirms our results of current experiments, namely that this compound is able to reduce weight losses during storage not only when introduced directly into the fruit but also when applied in the form of spraying; at the same time, this favourable effect eliminated can be almost completely with ascorbic acid applied jointly with maleic hydrazide. It is a generally known fact that maleic hydrazide stimulates the sugar metabolism (SCHOENE—HOFFMAN 1949, TERABUN 1958), which involves a considerable reduction of respiration (KIM—GREULUCH 1963) as a result of an enzyme inhibition (NAGUIB 1965). Within the cell maleic hydrazide is bound first of all to the cell wall and mitochondria, thus being able to take part directly in the control of the energetical processes (Hovanskaya—Povolotzkaya 1969 cit. RAKITIN *et al.* 1971).

The depression of development processes after maleic hydrazide treatments can be traced back — beside the antirespiratory effects of the compound — much more to an inhibiting effect on nucleic acid and protein synthesis (Suzuki 1966 cit. RAKITIN *et al.* 1971). It has already been suggested by some authors that the structure of maleic hydrazide being similar to that of the uracyl means an antagonistic character which could be demonstrated in cucumber and tomato plants (Povolotzkaya—Baskakov—Hovanskaya 1963 cit.

Table 2

Summarization of results graded by C.235 "Magyar kajszi", C.778 "Rózsabarack" and "Besztercei szilva" (lowest 3×1 → highest 3×13)

Concentration ppm		Storage loss weight %	Dry matter %	Ratio of invert sugar/ /acid
Control (tap water)		20	13	23.5
4000	AA	30	32	21
	MH	18.5	24	25.5
	AA+MH	22	26.5	19
2000	AA	15	21	19
	MH	10.5	13.5	30
	AA+MH	14	22.5	25
1000	AA	32	20	16
	MH	22	10	10
	AA+MH	20	10	24
500	AA	27	32.5	23
	MH	13.5	17	25
	AA+MH	28.5	31	12

Table 3

Correlations of storage loss, dry matter content and invert sugar/acid ratio in apricots and plums

Correlation		r — value		
		MK	RB	Bsz
Dry matter %	— Storage loss, weight %	+0.237	+0.158	+0.591*
Dry matter %	— Invert sugar/acid	—0.244	—0.359	—0.457
Invert sugar/acid	— Storage loss, weight %	—0.588*	—0.358	—0.586*

Results of grade correlations

Dry matter %	— Storage loss, weight %	+0.515*
Dry matter %	— Invert sugar/acid	—0.394
Invert sugar/acid	— Storage loss, weight %	—0.604**

* p = 10 %
** p = 5 %

Rakitin *et al.* 1971). Lobov (1971) pointed out that the incorporation of C^{14}-thymidine in DNA fractions can be inhibited with maleic hydrazide. In the course of testing compounds of similar structure it was found that riboflavin played an important role in the oxidation of maleic hydrazide in light (Andreae 1955 cit. RAKITIN *et al.* 1971).

Thus, in our recent investigations we have found that ascorbic acid is able to eliminate the effect of maleic hydrazide. It has an effect quite opposite to that of maleic hydrazide in many respects; while the latter inhibits the senescence of chlorophyll, is of anti-respiratory and anti-auxin character, the ascorbic acid, when applied with auxin, stimulates the decomposition of chlorophyll in peas (LAUDI 1961), increases the intensity of respiration and is synergetic with auxin (PILET 1961). Ascorbic acid is supposed to have a stimulatory effect on maleic hydrazide oxidation occurring under the influence of riboflavin, but it is not impossible either that it represses the riboflavine respiration becoming dominant under the influence of maleic hydrazide, and the normal process of respiration is restored again.

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VARIA

"SUBÁS KAPA"

Taxonomical place: *Nicotiana rustica* L. var. *brasilia* Schrank.

Origin: selected local variety.

Beginning of breeding: the first selection took place about fifty years ago.

State qualification: provisionally certified, improved variety, 1966.

Breeder: the first selection was made by Árpád Juhász; the variety was maintained in the seed plant of the Hungarian Tobacco Industry, Budapest.

General characterization: its considerable nicotine content makes it suitable for nicotine production; it is a high yielding, resistant variety (MÓGER—SZÜCS 1966).

Morphological description:

Root system: thickly branching adventive roots.

Shoot system: a thinly branched plant of more or less conic-cylindrical shape; its leaves falling close on each other make its shape seem compact.

Stem: thick, angular owing to the projecting ribs, strong, dark yellowish green. The plant is 50—90 cm high and about 2—3 cm thick at the base. The number of internodes is 20—23.

Foliage: the number of leaves is theoretically 18, but only 8—12 useful leaves are left on the stem. The leaves are petioled, their blade is rounded heart-shaped, crumpled, with a shiny surface and dark yellowish green colour. The leaves hang downward and fall close on each other (hence its name; "suba" is a wide sheepskin-coat reaching down to the heels). The leaves are covered with thick hairs. The petiole is fleshy, 10—15 cm long.

Inflorescence: moderately thick-set; cylindrical-roundish polychasium. The number of floral laterals is 16 on an average, and the number of flowers 230—240.

Flowers: stubby of an average length of 23 mm; calyx-tube stocky, half-egg shaped, lacinate; corolla-tube cylindrical, short and greenish yellow. Limbus round, indented by blunt lobes, undulate, 17 mm in diameter. Anthers yellow.

Fruit: round capsule of an average length of 10 mm, dehiscing at the apical part with a narrow slit. The number of capsules reaching maturation is 220.

Seed: small, brown; thousand-grain-weight; 27.7 cg.

Biological character:

Vegetation period: 80—90 days; flowering from the second decade of July.

Water requirement: responsive to groundwater; high yielding in moist soils (Kapás *et al.* 1965)

Resistance to diseases: fairly good.

Farm technology requirement:

Seeding: in the second half of March in a hot-bed.

Soil requirement: loam-, clay- and peat soils rich in nitrogen are preferred; the high humus content is important.

Productivity: the leaf yield is 15—17 q/ha, its nicotine content ranges between 2 and 5 per cent.

Region of cultivation: heavy soiled, high nutrient content tobacco growing areas of the Trans-Tisza, further the districts of Balatonnagyberek and Pápa (Móger—Szücs 1966).

Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen

Gy. Mándy

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CRITICAL BORON CONCENTRATION AND CALCIUM-BORON BALANCE AS RELATED TO THE BORON NEED OF SUGARBEET

Boron has a profound influence on the yield and quality of sugarbeet. Brandenburg (1931) working with the sugarbeet was the first to demonstrate clearly in the field that heart rot in sugarbeet was due to boron deficiency and could be prevented by boron application. A preliminary study has also indicated response to boron application in terms of beet yield at Pantnagar (India). In view of the increasing importance of this new crop, a need was felt that boron nutrition of promising sugarbeet varieties should be precisely studied. Thus, the present investigation was carried out with the following objectives: 1. To study the effects of added boron to soil on the quality and quantity of beet. 2. To study the relative susceptibility of sugarbeet varieties. 3. To determine the critical concentration of boron in leaf blade and to work out the calcium : boron ratio, which is helpful for the diagnosis of the boron requirement of sugarbeet.

Green house studies were conducted with seven promising sugarbeet varieties and four levels of boron (0, 0.5, 1.0 and 1.5 ppm) on the silty clay loam soil of Nainital Tarai (India). The characteristics of the different varieties of sugarbeet used in the experiment are as follows: L26700, KWS—E, Hh-Sokeri and L29619 diploid, open pollinated multigerm varieties, USH—9 a diploid, hybrid monogerm variety. Marocpoly and Resistapoly are anisoploid, hybrid multigerm varieties. The pH of the soil used in the experiment was 6.8. Exchangeable calcium and hot water soluble boron in this soil were 11.3 me. per 100 g and 0.78 ppm respectively. The processed soil was potted in polythene pots (24 × 25 cm), each containing 10 kg soil. A basal dose of 100 ppm nitrogen, 40 ppm phosphorus and 30 ppm potassium were given. Boron was supplied through reagent grade sodium borate. Three replications of each treatment were maintained. Six germinated seeds were planted in each pot. The seedling plants were thinned to two after three weeks of growth.

At the end of 95 days, the plants were harvested, washed with tap water, 0.01 N HCl and then with distilled water. The fresh root weight was recorded and then the roots were analysed for sucrose content by the cold extraction procedure of Brown—Zerban (1941). Dry ashing for leaf blade samples was done, then the ash was dissolved in 5 ml. of 1 N HCl and the volume was made up to 50 ml. with distilled water. These solutions were analysed for calcium by the volumetric method using E.D.T.A. (Heald 1965) and boron by the curcumin method (Jackson 1967).

Fresh beet weight, sucrose content and gross sugar yield. There was a significant increase in the fresh weight of roots upto 0.5 ppm added boron with the five varieties, namely KWS—E, Hh-Sokeri, L29619, Marocpoly and Resistapoly. Among these five varieties the fresh weight of the first three continued to increase, though non-significantly, even up to 1.0 ppm boron level and then it decreased with an increase in added boron above 1.0 ppm. But the fresh weight of the root in the last two varieties (Marocpoly and Resistapoly) decreased when boron was applied at the rate of 1.0 ppm or more (Table 1).

In the remaining two varieties L26700 and USH-9, there was no significant increase in fresh weight due to the 0.5 ppm boron level. At 1.0 ppm boron, the fresh weight in the variety L 26700 increased significantly and then decreased. Variety USH-9 showed a decrease in fresh beet weight at 1.0 and 1.5 ppm boron. The average fresh weight of all the varieties indicated that the increase in fresh root weight with 0.5 ppm was 22.3 per cent and with the 1.0 ppm boron level was 24.7 per cent over the control.

Besides influencing the beet yield, boron application also increased the sucrose content in beet resulting in higher sugar production. The application of 0.5 ppm boron increased the sucrose per cent and the gross sugar yield in all varieties but there was a decline in gross sugar yield at 1.5 ppm boron in most of the varieties (Tables 1 and 2). This is ascribed to the decrease in fresh weight of the beet with 1.5 ppm boron. It was also observed that 1.5 ppm boron increased the sucrose per cent in some varieties which may be attributed to their genetic characteristics. The increase in sucrose percent due to boron application had also been recorded by Hamence—Oram (1964). The effect of boron on the sugar content of the beet may be due to its possible role in sugar translocation (Gauch—Duggar 1954). A relationship between the complexing property of borate and sugar translocation has been implicated. Mcllrath—Polser (1956) have argued that boron prevents excessive polymerization of sugar at the site of sugar synthesis which leads to more sugar reserves in the beet.

Table 1

Influence of boron application on fresh weight and sucrose percentage of sugarbeet root

Varieties	Boron added to soil (ppm)							
	Fresh beet weight (g per pot)				Sucrose per cent			
	0	0.5	1.0	1.5	0	0.5	1.0	1.5
L26700	225.5	238.0	283.8	173.2	11.30	12.80	11.95	12.10
USH-9	196.0	221.3	211.5	173.5	11.10	12.20	13.00	13.90
Marocpoly	224.5	271.3	259.6	257.0	9.50	13.00	11.85	11.60
Resistapoly	214.4	248.5	227.5	178.1	11.75	13.80	12.30	12.00
KWS-E	219.7	267.5	277.6	251.5	10.30	12.30	12.80	11.10
Hh-Sokeri	202.0	306.5	321.6	280.1	10.65	11.75	12.10	13.00
L29619	186.0	242.7	248.9	224.0	10.90	12.50	12.70	14.10
Mean	209.7	256.5	261.5	219.6	10.78	12.62	12.38	12.54

C.D. at 5 per cent;

	Fresh beet weight	Sucrose per cent
Among boron levels	27.23	0.46
Among varieties	36.02	0.64
Boron levels × varieties	N.S.	1.18

N.S. = Not significant

Table 2

Influence of boron application on gross sugar yield of sugarbeet

Varieties	Boron added to soil (ppm)				Per cent increase of gross sugar yield in boron over no boron treatment
	0	0.5	1.0	1.5	
	Gross sugar yield (g per pot)				
L26700	25.14	30.46	33.92	20.96	+34.9
USH-9	21.74	26.49	27.42	24.12	+27.6
Marocpoly	21.33	35.27	30.68	23.39	+65.3
Resistapoly	25.21	34.29	27.98	21.37	+36.0
KWS-E	22.63	32.90	35.53	27.92	+56.9
Hh-Sokeri	25.53	36.01	38.93	36.41	+52.1
L29619	20.27	30.21	31.62	31.58	+55.7
Mean	23.12	32.30	32.30	26.53	—

C.D. at 5 per cent;

	Fresh beet weight	Sucrose per cent
Among boron levels	4.58	—
Among varieties	4.97	—
Boron levels × varieties	N.S.	

Susceptibility of sugarbeet varieties to boron deficiency. The susceptibility of sugarbeet varieties to boron deficiency may be explained on the basis of the increase in gross sugar yield. Variety Marocpoly was found to be most susceptible to boron deficiency and it recorded a 65.3 per cent increase in the gross sugar yield with boron over the control. Variety USH-9 was the least susceptible to boron deficiency and it produced only 27.6 per cent more sugar yield (Table 2). The seven sugarbeet varieties followed the following order with respect to their susceptibility to boron deficiency; Marocpoly > KWS-E > L29619 > Hh-Sokeri > Resistapoly > L26700 > USH-9.

The high susceptibility of Marocpoly to boron deficiency may be due to its genetic constitution. The varieties Marocpoly and Resistapoly were anisoploid i.e. a mixture of diploid, triploid and tetraploid. The differential nature of the two anisoploid varieties in susceptibility

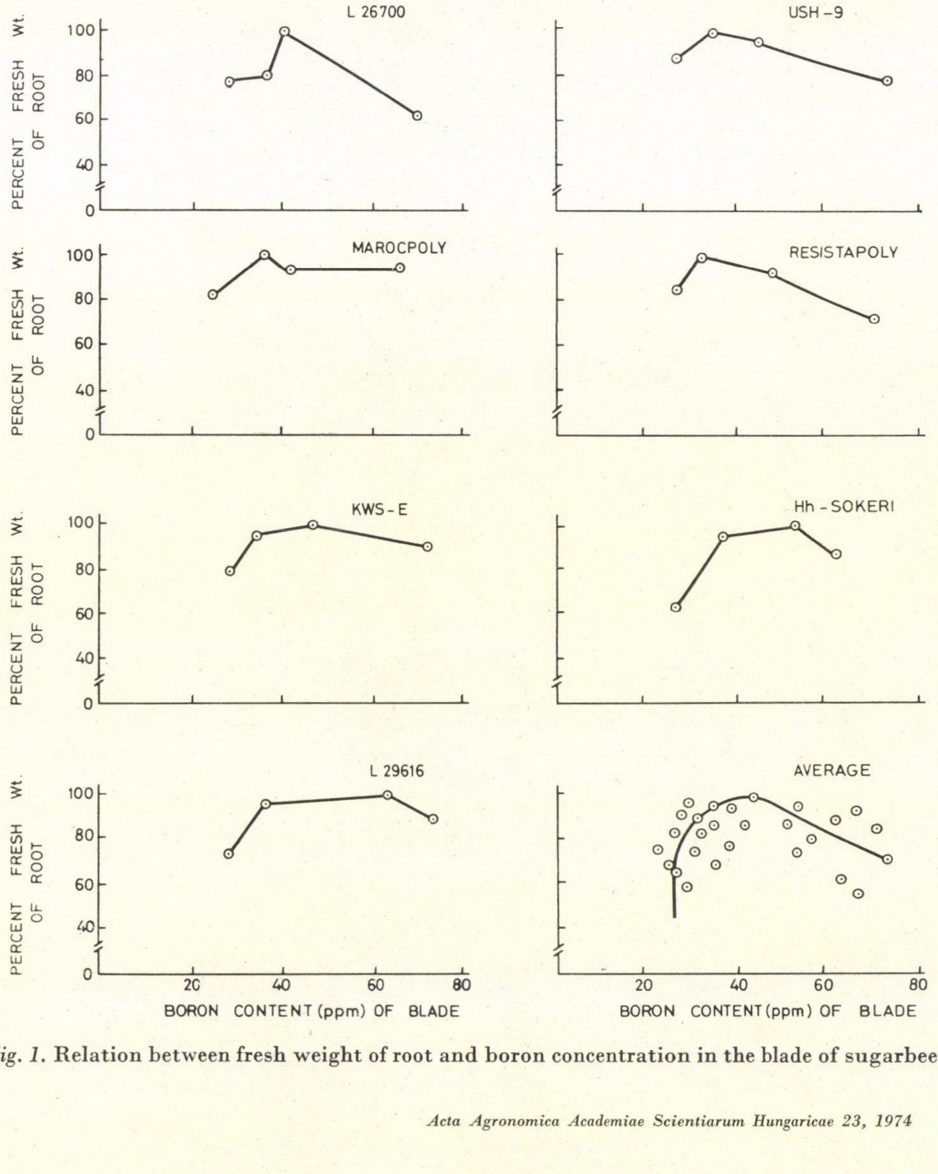

Fig. 1. Relation between fresh weight of root and boron concentration in the blade of sugarbeet

to boron deficiency can be explained on the basis of their genetic constitution. There might be certain genes, constituting the genetic make up of the variety, which might be responsible for the differential response of the two anisoploid varieties to boron deficiency.

Critical boron concentration in the blade. The chemical analysis of plant tissues as a measure of assessing the nutrient requirements of the plant has often been based on the concepts of the plant nutrient calibration curve and critical nutrient concentration (Ulrich 1948).

The plant nutrient calibration curve relates plant growth to the nutrient concentration of a specific plant part, as for example, when the fresh or dry weight of tops and roots are plotted as a function of the boron concentration of the petioles or blades of either young, recently matured or old leaves. When plotted in this manner, the plant nutrient calibration curve postulates the existence of three zones of nutrition; zone of deficiency, zone of adequacy, transition zone. It is the transition zone that contains the critical nutrient concentration, defined as that concentration of the element in the plant tissue at which a significant reduction in yield of about 5 to 10 per cent from the maximum is observed (Ulrich 1962). Within the transition zone both nutrient concentrations and yields increase simultaneously as more nutrients are absorbed by the the plant.

The critical boron concentration for the growth of different sugarbeet varieties varied from 28 to 39 ppm in the mature leaf blades. This range approximates the breaking point of the curve. Below this range of values, plants tend to be deficient in boron and above they are well supplied. The lowest critical concentration (28 ppm) was in varieties USH-9 and Resistapoly whereas the highest critical boron concentration was in the variety L 26700. The average critical boron concentration of all the varieties was 34 ppm (Fig. 1).

Calcium-boron relationship in sugarbeet. The relationship between calcium and boron was observed by Reeve—Shive (1943). They observed that when plants were supplied with increasing amounts of calcium, they required more boron to prevent deficiency and with high

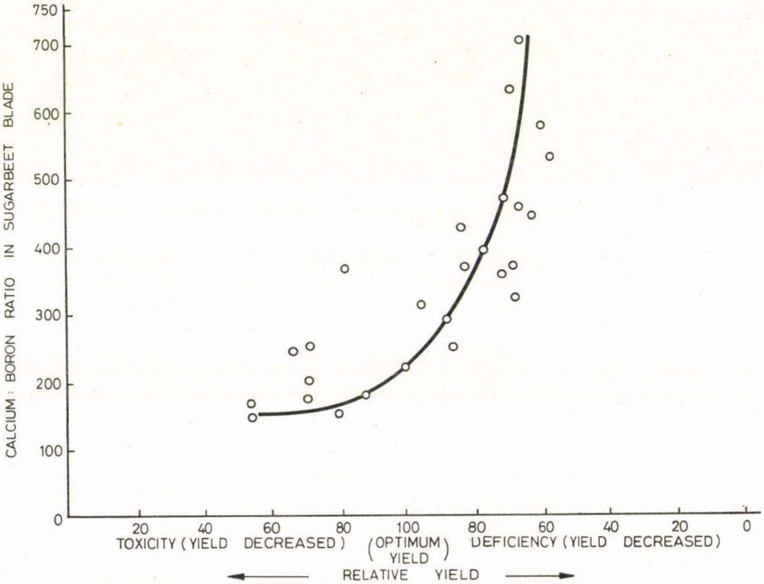

Fig. 2. Relation between calcium boron ratio of blade and relative yield (per cent fresh weight of root)

amounts of calcium, plants could withstand larger amounts of boron without any toxic effect. Results in the experiment revealed that the increase in added boron to the substrate decreased the calcium : boron ratio in the leaf blade. An attempt had been made to determine the relationship between the yield and the calcium : boron ratio in the leaf blade. The curve obtained between the relative yield (percent fresh beet weight) and calcium : boron ratio in the blade on an equivalent basis indicated that the ideal ratio between the calcium and boron for maximum yield (100 per cent) was 220 to 1. It would appear that soils known to be high in available calcium should be suspected of producing boron deficient plants unless proved otherwise, as the plants grown therein would have a high calcium : boron ratio in the plant ash.

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Prepared at the G. B. Plant University of Agriculture and Technology, Pantnagar.

R. R. Singh, M. S. Gangwar

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NON-SYMBIOTIC NITROGEN FIXATION BY AZOTOBACTER AS INFLUENCED BY SOIL SALINITY

Saline soils occupy vast areas in U.A.R. especially in the Northern Regions of the Nile Delta, where the soils contain about 3000 to 6000 ppm. total soluble salts including 1800 to 2400 sodium chloride.

Reclamation of such soils has been found to be more easier than that of alkali soils which need beside leaching additional amounts of gypsum and organic matter (Taha—Mahmoud—Ibrahim 1964, 1965).

The aim of the present investigation is to study the distribution of *Azobacter* as influenced by soil salinity, and to find out how different concentrations of sodium chloride can affect the rate of nitrogen fixation by certain strains of *Azotobacter.*

This is found to be of great interest, since many investigators have drawn the attention to the importance of non-symbiotic nitrogen fixation and to the presence of high densities of *Azotobacter* in Egyptian fertile and deteriorated soils (ISHAC 1958, MOBAREK 1960, ABDEL-HAFEZ 1962, HOSNY 1962, EL-SAID 1963, VANCURA—ABD-EL MALEK—ZAYED 1965, TAHA *et al.* 1965 and MAHMOUD—TAHA—IBRAHIM 1974).

Nitrogen fixing organisms, however, were known to be remarkably resistent to the action of high concentration of soluble salts in the soil (LIPMAN—SHARP 1912, BARNES—ALI 1917, HOSNY 1962 and BABALS 1965).

In this respect, sodium salts have been found to be unnecessary for the growth and activities of *Azotobacter*, and excessive amounts of them might depress fixation of nitrogen (ISWARAN—ABHISWAR SEN 1958, ISWARAN *et al.* 1965).

On the other hand, a large number of analyses showed that the presence of high concentration of salts in the soil only rendered *Azotobacter* inactive or slightly active. They, however, started again their activities with the removal of salts by irrigation or leaching during reclamation (GREAVES—CARTER—LUND 1922, TAMHANE—KRISHNA 1931 and TAHA—MAHMOUD—IBRAHIM 1964, 1965).

20 soil samples were collected from different parts from the Northern Region of the Nile Delta. They were examined bacteriologically within 24 hours, then air dried, ground, sieved through a 2 mm sieve and kept in glass bottles for the further determinations.

Bacteriological. *Azotobacter* were counted on Base medium 77 (ALLEN 1961) using the dilution frequency method, after which the most probable numbers were calculated on the oven dry basis using Hoskins' tables.

Azotobacter cultures were isolated according to ALLEN (1961). The isolates were examined for morphological, cultural and biochemical characteristics and isolates numbers 15, 17 and 27 were used in the present investigation.

Chemical. Salt contents were determined by extraction with CO_2-free distilled water (dilution 1 : 5) and evaporation of a suitable aliquot to a constant weight. Ph values were determined with a Bekman glass electrode in soil suspension (1 : 2.5). Walkely and Black wet digestion method was used for the determination of organic carbon and Kjeldahl method was used for the determination of total nitrogen (JACKSON 1958). Somogyi method (A.O.A.C. 1950) was used for sugar determinations.

In the first part of this investigation, a statistical correlation was calculated between *Azotobacter* populations and salt contents of 20 soil samples collected from the Northern Region of the Nile Delta (Table 1).

This correlation was found to be significantly negative ($r = -0.84$). The highest count of *Azotobacter* was counted in the more fertile soil (T.S.S. = 0.44%), and the lowest count was recorded in the more saline soil (T.S.S. = 9.61%) in the present investigation. This, however, shows clearly how salinity could inhibit the growth and proliferation of *Azotobacter.*

The growth of *Azotobacter* was found also to be affected with soil pH to a great extent as shown in Table 2.

The highest counts were obtained in this experiment in soils having pH values more than 7.9. *Azotobacter* is well known to be highly sensitive to soil acidity with an optimum at pH 7—8. However, WAKSMAN (1961) stated that *Azotobacter* usually cannot develop in a soil having a pH of less than 6.0. In the present investigation, the maximum count of *Azotobacter* was recorded at pH 8.2, and the minimum count was recorded at pH 7.3.

In the second part of this investigation, it was found of interest to study the effect of leaching of the toxic salts on the growth and proliferation of *Azotobacter.* Soils numbers 3 and

Table 1

Azotobacter counts, salt contents and pH values of the tested soils

Soil No.	Location	T.S.S. %	pH	*Azotobacter* in million/g dry wt. of soil
1	Mehalet Mosa	0.44	8.2	4.666
2	,,	3.44	7.8	1.525
3	Shalma	5.20	7.7	1.820
4	,,	9.61	7.3	0.038
5	Bahr El-bakar	4.84	7.9	0.818
6	,, ,,	5.42	7.8	0.805
7	,, ,,	6.84	7.7	0.753
8	,, ,,	2.10	7.9	2.411
9	,, ,,	8.32	7.6	0.191
10	,, ,,	5.10	7.8	0.462
11	,, ,,	6.82	7.7	0.577
12	Aboul-Akhdar	4.13	7.9	0.948
13	,,	8.12	7.6	0.240
14	,,	4.16	7.9	1.301
15	Sakha	1.18	8.1	2.422
16	,,	0.68	8.2	4.301
17	Menshat Selim	6.20	7.6	0.430
18	,, ,,	8.10	7.3	0.380
19	,, ,,	7.62	7.4	0.146
20	,, ,,	9.18	7.2	0.087

Table 2

Effect of soil pH on the growth of Azotobacter

pH	Count of *Azotobacter* in million/g dry wt. of soil
<7.3	0.037—0.380
7.3—7.6	0.078—0.430
7.6—7.9	0.191—1.301
7.9—8.2	0.948—4.666

13 were used for this study. One kg of soil was placed in a glass tube with a narrow neck, in which a piece of glasswool was put to facilitate the leaching process. The glass tubes were arranged under the laboratory condition, where the soils were submerged with tap water 3 times. Sampling was carried out before the following application of water, at 2, 4 and 8 weeks. Fresh samples were used for the counting of *Azotobacter*, and air dried soils were used for chemical determinations.

As shown in Table 3, a gradual decrease was recorded in the salt contents of both soils. This was expected due to the leaching process. However, the highest amount of salts were leached after the first washing. These were found to be 60 per cent and 68 per cent of the total leached salts in the two reclaimed soils respectively.

Table 3

The effect of leaching of saline soils on T.S.S., pH values, total nitrogen and counts of Azotobacter

Soil No. 3					Soil No. 13				
Time in weeks	T.S.S. %	pH	T.N. %	*Azotobacter*	Time in weeks	T.S.S. %	pH	T.N. %	*Azotobacter*
0	5.20	7.7	0.085	1.820	0	8.12	7.6	0.102	0.240
2	2.48	7.9	0.079	4.620	2	3.15	7.9	0.090	3.537
4	1.62	8.0	0.080	5.046	4	1.74	7.9	0.090	3.899
8	0.68	8.0	0.080	4.014	8	0.79	8.1	0.098	5.114

Azotobacter in million (g. dry wt. of soil).

A somewhat gradual increase in soil pH was also recorded during the leaching process, pH values reached 8.0 and 8.1 in the two soils, respectively. This could be attributed to elimination of the effect of salt concentration, giving the opportunity for the exchangeable and soluble sodium to react by producing sodium carbonate and bicarbonate and increasing the pH value of the soils (Taha—Mahmoud—Ibrahim 1965).

The leaching of salts has caused a significant increase in *Azotobacter* populations in both soils. The maximum increase was recorded after 2 weeks, accompanied by the maximum loss of salts. This shows clearly the toxic effect of salts on the growth and proliferation of *Azotobacter*. However, the activities of *Azotobacter* were negligible and no increase was detected in the total nitrogen content of both soils. On the other hand, the total nitrogen content showed a somewhat significant decrease, which could be attributed to the leaching of soluble nitrogen.

Later, it was found of interest to study, in pure cultures, the effect of different concentrations of sodium chloride on the nitrogen fixation process. Cultures number 15, 17 and 27 were, however, used in this experiment. The first two strains were isolated from a highly saline soil, N° 13, and the third strain from the soil N° 1, having the lowest concentration of salts. The various strains were cultured in 25 ml of Burk's solution (1930) with traces of molybdenum and boron in Erlenmeyer flasks of 500 ml capacity, and to which different concentrations of sodium chloride up to 4% were added. Three replicates were carried out, and the flasks were incubated at 28°C, for 15 days. Analyses were carried out in duplicates, and the average results expressed as mg nitrogen fixed per 1 g of carbon utilized are shown in Table 4.

It is evident from the present data that nitrogen fixation was inhibited by sodium chloride. The rate of inhibition depends to a great extent on the source of the cultures used.

That is, cultures isolated from soils of higher salinity showed a lower rate of inhibition than that isolated from non-saline soil.

The critical concentration at which *Azotobacter* showed a significant decrease in their abilities for nitrogen fixation varied also with the different strains. This was found to be at the concentrations of 1.5%, 0.8% and 0.4% for the cultures number 15, 17 and 27, respectively, denoting that strains isolated from saline soils could be affected to a lesser extent with higher concentrations of sodium salts than that isolated from non-saline soils.

Table 4

Effect of sodium chloride on nitrogen fixation by the different strains of Azotobacter

Sodium chloride, %	mg nitrogen fixed/g carbon utilized		
	Strains No.		
	15	17	27
Control	12.8	11.6	11.8
0.05	13.0	11.4	11.8
0.10	13.6	13.8	12.0
0.20	12.8	12.0	11.2
0.40	12.2	11.9	2.9
0.60	12.0	12.3	2.6
0.80	12.4	8.4	—
1.00	12.0	8.0	—
1.50	5.8	8.0	—
2.00	2.2	0.3	—
3.00	1.2	—	—
4.00	0.2	—	—

The data also showed that lower concentrations of sodium chloride up to 0.1% had a somewhat significant effect in increasing nitrogen fixation by the 3 cultures of *Azotobacter*. This, however, showed that sodium salt may play a role in the process of nitrogen fixation.

Azotobacter were found to be present at higher densities in Egyptian fertile soils than observed anywhere else (Ishac 1958, Mobarek 1960, Abdel-Hafez 1962, Taha *et al.* 1965). Similarly, their occurrence at relatively high densities in saline and alkali soils of Egypt was found to be very interesting (Hosny 1962, El-Said 1963, Taha—Mahmoud—Ibrahim 1964, 1965).

In the present investigation, a significant negative correlation ($r = —0.84$) was calculated between salt concentrations and counts of *Azotobacter* in 20 saline soils varying in their salt contents. This shows the toxic effect of salts on the growth and proliferation of *Azotobacter*.

The reclamation of such soils by washing and leaching the excess salts is expected to increase the growth and proliferation of *Azotobacter*. This is what has exactly happened in this investigation. Two saline soils were washed by water, and salts leached out, which significantly increased *Azotobacter* population in both soils. The maximum increase was parallel with the maximum decrease in the salt contents. This denotes that the presence of high concentration of salts in the soil only rendered *Azotobacter* inactive or slightly active. This was found to be in agreement with GREAVES—CARTER—LUND (1922), TAMHANE—KRISHNA (1931), ISWARAN—ABHISWAR SEN (1958) and TAHA—MAHMOUD—IBRAHIM (1964, 1965), who found that the removal of toxic salts by irrigation or leaching during reclamation significantly increased *Azotobacter* activities.

One should also add that the reclaimed soils must be amended with organic nitrogenous materials, since the reclamation of such soils causes, as a result of leaching, a significant decrease in the soluble nitrogen contents.

Azotobacter cultures isolated from different soils have also showed a different response towards sodium chloride. 3 strains were cultured in broth medium containing different concentrations of sodium chloride ranging from zero to 4 per cent. In general, the nitrogen fixation process has been inhibited by sodium chloride. The critical concentration at which *Azotobacter* showed a significant decrease in the ability for nitrogen fixation was found to be at 1 per cent. This, however, depends upon the source of the strains. *Azotobacter* isolated from the most saline soil was found to be more resistant to the inhibition action than that isolated from a slightly saline soil. In other words, the concentration of sodium chloride which had inhibited the nitrogen fixation process was higher for *Azotobacter* isolated from soil containing more soluble salts than for the organism isolated from less saline soils.

Hence, it can be concluded that with the exception of *A. halophylum* and those of marine habitat, which are probably the varieties of the former, *Azotobacter* in common soils are only inactivated by high concentrations of salts, but with the lowering of the concentration of salts they again start showing normal activities.

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Prepared at the Faculty of Agriculture, Al-Azhar University, Cairo.

A. N. IBRAHIM

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PHYSIOLOGY OF HOST-PATHOGEN INTERRELATION WITH RESPECT TO PROTEIN LEVELS

Recently it has been demonstrated in the international literature, that the purin-type synthetical cytokinins in the N^6-derivatives (kinetin, benzyladenine) (**Osborne** 1962), as well as the natural cytokinins (zeatin, dihydrozeatin) have remarkable biological effectivity on the stimulation of the synthesis of nucleic acids and protein (**van Overbeek** 1966). It has likewise been confirmed by us, that the non purin-type benzimidazole (**Pozsár**—**Király**—**El Hammady** 1967), and the pyrimidine type 6-methyluracil (pseudothymine) (**Pozsár**—**Matolcsy** 1968) have a similar biological activity on the synthesis intensity of protein and its contents respectively.

On the other hand for a large number of purins (9-methyladenine, 9-methylbenzyladenine, 7,9-dimethylbenzyladenine), pyrimidines (6-methyluracil, 6-azauracil, 6-azathymine and the potential precursors for the 6-methyluracil), benzimidazoles (N,N'-dimethylbenzimidazole, 2-aminobenzimidazole, Benlate, Thiabendazole) (**Pozsár** 1972) labelled with radioactive carbon on the methyl groups, we have shown, that these compounds have a demethylation process in the metabolism, and on effectivity on the protein synthesis. In this paper the biological activity of cytokinins and resistance factors (systemic fungicides) have been demonstrated first of all by changing different protein fractions under the effect of infection and varying the cases of treatment with bioactive compounds.

The experiments were performed with wheat (*Triticum aestivum* L.) Bezostaya-1, and Bánkuti 1201 varieties, and with bean (*Phaseolus vulgaris* L.) Pinto variety.

In the case of Bezostaya-1 wheat leaves the intensity of protein synthesis was investigated with the aid of radiocarbon labelled glycine-1-^{14}C, in the different protein fractions after incorporation at the following short intervals; 3, 6, 12, 18, 24 hours. The leaf protein was fractionated with 0.5% NaCl, 4 N H_4NOH, and 20% NaOH, and the synthesis intensity was characterized on the basis of the radioactivity of different fractions. The specific activity of the applied glycine-1-^{14}C was 26 m.ci./mmole, and the activity of the external solution 0.5 micro ci./ml. The leaf segments were floated for 4 hours, and the radioactivity was measured with the aid of the liquid-scintillation method.

In the following experiments the fractionation of the leaf protein was performed by Sephadex gel chromatography, and the different fractions were characterized by their molecular weights. The primary leaf growth and senescence of Pinto bean could be demonstrated by the molecular weight of the different protein fractions. The first (12,000 molecular weight) and second (24,000 molecular weight) fractions of protein were probably analogous with the soluble (0.5% NaCl) fractions. The ratio and changes of the different protein fractions in the surface growth of bean leaves was investigated for the evaluation from the pathophysiological respect

(Pozsár—Horváth—Lehoczky—Sárospataki 1969), and for determining the biological effectivity of the imidazole derivatives.

The host-parasite relationship may be characterized by separating the protein from the healthy and infected wheat and bean leaves. The pathological effect on the protein level is related to the senescence of the leaf development, and the action of different systemic fungicides, in the cases of obliged parasites.

The effect of adenine-type cytokinins and the benzimidazole derivatives was compared with the synthesis intensity of the different protein fractions. The primary half leaves of seedlings were treated once a day for 6 days, and in the second week — before the separation — the plants remained in the untreated form (Pozsár—Király—El Hammady 1967).

The pathophysiological investigation on the protein level presents a possibility for the interpretation of the biological effectivity of the cytokinins and the hormone-like biological activity of the benzimidazole type systemic fungicides.

The intensity of protein synthesis in the leaf tissues can be well characterized by the incorporation of the radiocarbon labelled amino acids (glycine-1-^{14}C) into the different fractions, with the method of floating for various lengths of time, as described in an earlier paper (Pozsár 1971). With the aid of the partial fractionation of the leaf protein in the Bezostaya-1 wheat variety we have succeeded in proving directly that the highest radioactivity per unit time accumulates in the soluble protein fraction, as shown in Table 1. In the different protein fractions the radioactive carbon labelled amino acid incorporated at varying rates, which are the consequence of the synthesis intensity. The radioactivity of the insoluble protein fraction could be demonstrated only much later and was lower than in the soluble fractions.

The different molecular weight protein fractions in the development of the leaf varied with a similar tendency. On the basis of the data in Table 2 it can be seen, that there was a relatively higher rate in the lower molecular weight protein fractions, than in the bigger ones in the first phase of leaf development. The lower molecular weight protein fractions of the bean leaf become relatively diminished, while the rate of the highest molecular weight fractions related to the earlier phases increases. The first and second fractions in the processes of senescence decreased by 60 and 43 per cent, respectively, the middle fractions increased by 20 and 46 per cent respectively, at the same time the investigated highest fraction increased by 53 per cent related to the original phase. The decrease of the lower molecular weight fractions showed a very strong positive correlation with the phytopathological virulence of the sensitive host plants.

Table 1

Synthesis intensity of different soluble protein fractions of Bezostaya 1 wheat leaves, tested by the incorporation of 1000 c.p.m./100 mg fresh weight radioactive carbon labelled glycine-1-^{14}C in

Incorporation in hours	Solubility in		
	0.5% NaCl	4 N N$_4$NOH	20% NaOH
3	12.5	—	—
6	20.1	3.1	—
12	34.2	13.6	1.4
18	43.7	22.0	10.6
24	51.3	29.3	16.2

Table 2

Changes in the different protein fractions of Pinto bean leaves, with 21.2% protein content, characterized by the molecular weight, and the indication of the virulence of U. phaseoli

Development of leaves in days	Protein molecular weight (1000×)					Virulence
	12	24	36	120	400	
4	3.7	5.9	5.0	5.2	1.3	not at all
8	3.5	5.1	5.1	6.0	1.4	very difficult
12	3.2	4.0	5.6	7.2	1.2	difficult
16	2.6	3.1	5.7	7.3	2.4	easy
20	2.1	2.4	6.0	7.6	2.0	very easy
Ranges in the percentage of fractions, related to the first phase	−60	−43	+20	+46	+53	

Table 3

Effect of infections on the different molecular weight fractions of Bánkúti 1201 wheat variety, with 13.4 per cent protein content, and of Pinto bean variety, with 21.2 per cent protein content, at the sporulation phases expressed by the infection induced changes of the different protein fractions as a percentage

Fractions in 1000 molecular weight	Bánkúti 1201 wheat variety			Pinto bean variety		
	Healthy	Infected by *P. graminis*	Changing of fraction in %	Healthy	Infected by *U. phaseoli*	Changing of fraction in %
12	2.1	1.4	66	4.0	2.3	57
24	1.7	1.1	64	3.2	2.8	87
36	3.8	5.0	131	5.6	6.4	114
120	4.3	4.5	104	7.2	7.5	104
400	1.5	1.4	93	1.2	2.2	183

On the other hand in the later stage of the infection the change in the rate of the different fractions had a similar mode of action as natural senescence, as shown in Table 3. According to the data obtained, in the *Uromyces phaseoli* infected twin leaves the 12,000 fraction decreased to 57 per cent, and in the case of *Puccinia graminis f. sp. triticii* infected wheat leaves the intensity of level diminution rose to 66 per cent related to the healthy controls while the higher fractions increased under the effect of infection, at the sporulation phases.

According to the results of the experiments carried out with the new purin, pyrimidine and benzimidazole type synthetic plant hormones, bioactive compounds and resistance factors we produced a more analogous biological effectivity, compared to the earlier phase of development of the leaves. The level of lower molecular weight fractions increased primarily in the leaves of the host plants (Table 4). The level increase showed +37% and +22% in the average values, and the mean decrease of the 400,000 molecular weight fraction was 77 per cent related to the untreated control, in the case of the Pinto bean variety.

Table 4

Effect of one week treatments of cytokinins and resistance factors (benzimidazole type systemic fungicides) on the changing of different molecular weight protein fractions one week after the end of treatments, at 21.1 per cent protein content, related to the dry weight of Pinto bean leaves

Bioactive compounds	Applied ppm	Protein molecular weight (1000×) 12	24	36	120	400
Control	—	3.2	4.0	5.1	6.2	2.6
Kinetin	50	4.5	5.1	4.7	6.0	0.8
Benzyladenine	30	4.9	5.0	4.6	5.8	0.7
6-Methyluracil	200	4.2	4.8	5.1	5.7	1.3
Benzimidazole	200	4.3	5.0	5.0	5.8	1.0
5,6-Dimethylbenzimidazole	200	4.2	4.7	5.5	6.0	0.7
2-Aminobenzimidazole	200	4.7	5.1	5.2	5.8	1.3
"Benlate"	200	4.1	5.0	5.3	6.0	0.7
"Thiabendazol"	200	4.4	5.1	5.4	5.6	0.6
Mean value related to the control		4.4	4.9	5.0	5.8	0.88
Difference of mean value as a percentage from the control		+37	+22	−2	−7	−77

The effect of synthetic cytokinins and the resistance factors (systemic fungicides) at the protein level produced a remarkable rate of change with the different fractions, first of all in the treated leaves the increased level of lower molecular weight (soluble) can be attributed to the protein fractions. The significant change of different fractions in the protein level is perhaps not independent of the increase of immunological active specificity fractions.

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Prepared at the Agrobotanical Institute, Tápiószele.

B. I. Pozsár

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EFFECT OF NITROGEN FERTILIZATION ON THE PROTEIN CONTENT AND AMINO ACID COMPOSITION OF WHEAT GRAIN

The increase of protein content in wheat grain owing to nitrogen fertilization has been reported by several workers (HALLIDAY 1960, PFAFF 1955, LINSER 1960, PRIMOST 1960 and AUSTIN *et al.* 1971). From the nutritional point this increase is important provided there is no imbalance in the composition of the various amino acids in the proteins. Studies on these aspects are necessary particularly in view of the observations made by several workers that some of the essential amino acids decrease when the protein content increases due to nitrogen fertilization. A negative correlation between the lysine and total protein content of wheat grain has been reported by GUNTHARDT—MCGINNIS 1957, LAWRENCE *et al.* 1958, MCDERMOTT—PACE 1960, SIMMONDS 1962, BHAID *et al.* 1969 and AUSTIN *et al.* 1972. LARSEN—NIELSEN 1966 found decreases of lysine, threonine and valine and increases of glutamic acid, proline and phenylalanine in wheat protein when nitrogen was applied in the form of ammonium sulphate. On the other hand HUCKLESBY *et al.* 1971 reported that the quality of grain with reference to amino acid composition was not affected with split doses of nitrogen on some late spring wheats. These divergent results appear to point out possible varietal differences with regard to the turnover of amino acids with nitrogen fertilization. In this work two high yielding wheat varieties have been studied with regards to the effect of nitrogen fertilization on protein and amino acid composition.

Grains of wheat varieties Kalyansona and Heera grown under 0, 80 and 160 kg.n/ha during the crop season 1971—72 were tested for total protein and amino acid composition. Total protein ($N \times 5.7$) was determined according to the usual macro-Kjeldahl method. The amino acid composition was studied using a Technicon automatic amino acid analyzer. A defatted sample containing 5 mg protein was hydrolysed by refluxing with 6 N HCl for 22 hours. After the removal of acid by evaporation under reduced pressure, the residue was dissolved in 2 ml of citrate buffer of pH 2.875. Aliquot measuring 0.4 ml was used for the determination with the analyzer according to the method described by MOORE—STEIN (1954).

The data for protein and amino acid composition in Kalyansona and Heera are given in Tables 1 and 2 respectively. The protein content of the grain increased markedly in both the varieties with the application of 80 as well as 160 kg N/ha. This increase in the case of Heera was at a constant rate and of higher magnitude than that found in Kalyansona. With 160 kg N/ha it had given 48.4 per cent increase over the control (N_0) while in Kalyansona the increase was only 28.7 per cent.

The data for amino acid composition show that, in general, glutamic acid, aspartic acid, alanine, valine, leucine and phenylalanine increased while proline, glycine, tyrosine and lysine decreased in the protein produced after nitrogen fertilization. The data furthermore show that the two varieties differed markedly with regard to the turnover of some of the amino acids in the protein due to nitrogen fertilization. Compared to Kalyansona, Heera had shown markedly higher increases in aspartic acid, valine, leucine, phenylalanine and histidine particularly with 160 kg N/ha. In addition, threonine decreased in Heera while it was unaffected in Kalyansona. Arginine and histidine showed a decreasing trend in Kalyansona.

The data for the different essential amino acids expressed per 1 g of total essential amino acids (mg) g total essential amino acid given in Table 3 clearly show that lysine tended to decrease in both the varieties with nitrogen fertilization. Heera in addition showed marked decreases in threonine content.

Table 1

Protein percentage and amino acid composition in Kalyansona
(amino acid; g/100 g protein)

Amino acid	Nitrogen kg/ha		
	Control (N_0)	80 kg N	160 kg N
Aspartic acid	6.63	6.40	6.88
Threonine	2.93	2.93	3.04
Serine	4.39	4.99	4.34
Glutamic acid	29.16	32.72	33.76
Proline	10.83	9.88	9.71
Glycine	4.24	3.81	3.88
Alanine	3.14	3.36	3.63
Valine	5.26	6.23	5.78
Cystine	1.86	1.82	1.92
Methionine	1.92	1.95	1.96
Isoleucine	3.64	3.38	3.66
Leucine	6.01	7.69	6.77
Tyrosine	3.21	3.08	2.96
Phenylalanine	4.35	4.60	4.71
Ammonia	2.86	2.42	2.40
Lysine	2.93	2.75	2.78
Histidine	2.36	2.01	2.88
Arginine	4.55	4.37	3.80
Tryptophane*	—	—	—
Protein %	10.88	12.54	14.00

* Not determined.

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*

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A. Austin, V. P. Ahuja

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Table 2

Protein percentage and amino acid composition in Heera
(amino acid; g/100 g protein)

Amino acid	Nitrogen kg/ha		
	Control (N$_0$)	80 kg N	160 kg N
Aspartic acid	5.48	6.80	6.65
Threonine	3.66	2.85	3.05
Serine	5.05	4.01	5.56
Glutamic acid	30.43	32.73	33.34
Proline	10.50	10.66	9.09
Glycine	4.98	4.71	4.12
Alanine	3.16	3.64	3.42
Valine	4.54	5.66	6.20
Cystine	1.59	1.40	1.57
Methionine	1.75	1.40	1.73
Isoleucine	3.25	3.33	3.20
Leucine	6.40	7.25	7.75
Tyrosine	3.88	3.45	3.48
Phenylalanine	4.78	5.18	5.44
Ammonia	2.91	2.08	2.17
Lysine	2.97	3.02	2.80
Histidine	2.32	2.34	2.86
Arginine	4.16	5.25	4.18
Tryptophane*	—	—	—
Protein %	10.95	13.36	16.25

* Not determined.

Table 3

Amount of essential amino acids in Kalyansona and Heera expressed in terms of total essential amino acids
(mg/1 g total essential amino acid)

Essential amino acid	KALYANSONA			HEERA		
	Control (N$_0$)	80 kg N	160 kg N	Control (N$_0$)	80 kg N	160 kg N
Lysine	107	93	96	108	105	92
Phenylalanine	160	155	164	175	180	180
Methionine	71	66	67	64	47	57
Threonine	107	99	105	133	99	101
Isoleucine	134	114	127	118	116	106
Leucine	222	260	239	234	252	256
Valine	194	210	201	165	197	205
Tryptophane*	—	—	—	—	—	—

* Not determined.

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SEASONAL VARIATIONS IN THE MINERAL CONTENT OF WESTERWOLDS, TETRONE AND NORMAL RYEGRASS VARIETIES IN COMPARISON WITH BALADI 16 BARLEY VARIETY

The importance of mixing grasses such as ryegrass and barley with legume forages is progressively increasing in the U.A.R. One of the main studies in this field is that concerned with the mineral composition of these grasses.

It was found that as the grasses mature, the protein and ash contents fall while the crude fiber content rises (**Wilson** 1889, **Wolff** 1895, **Waite—Sastry** 1949, **Dijkstra** 1957). **Fagan** (1928) studied the mineral composition of Italian ryegrass at different stages of growth. The values obtained were 3.07, 1.94 and 1.10% for nitrogen, 0.88, 0.45 and 0.37% for phosphorus (P_2O_5) and 3.75, 3.17 and 2.20% for potassium (K_2O) at 2, 6 and 10 weeks of growth respectively. **Norman** (1933) working with barley found that both ash and proteins showed an initial increase followed by a steady fall as development proceeded. **Fraps—Fudge** (1945) investigated 64 species of range pasture grasses and pointed out that protein decreased rapidly from the young to the intermediate to the mature stage of growth. They added that the relative effect of maturity upon the protein content varied widely with different species. They also found that the phosphoric acid ranged from 0.69 to 0.32% in young grasses and from 0.44 to 0.14% in mature ones. **Homb** (1952) found a correlation between the percentage of phosphorus and the stage of growth in grasses, namely, that it falls with increasing age. Similar results were obtained by **Thomas** *et al.* (1952) and **Nommik** (1955). Significant differences were found in protein between species and between stages of growth as reported by **Heinrichs—Carson** (1956) working with nine grasses including Mandan wild ryegrass.

The present work was carried out to study the seasonal variations in the mineral composition of some ryegrass varieties in comparison with barley.

A pot experiment was conducted in the Faculty of Agriculture, Ain Shams University, U.A.R. to study the mineral composition of three ryegrass varieties namely Westerwolds, Tetrone and Normal in comparison with the Baladi 16 barley variety during their growth and development.

Detailed information concerning experimentation and sampling were mentioned in a previous paper (EL-KADI *et al.* 1970).

For chemical analyses, the dried material was used after being ground to a fine powder. The total nitrogen was determined using the modified micro-Kjeldahl method as described by PREGL (1945). Phosphorus was estimated colorimetrically according to the method already mentioned by SNELL—SNELL (1954) using a Viscomat Garat Colorimeter. Potassium was determined photometrically as described by BROWN—LILLELAND (1946) using a Carl Zeiss Jena flame photometer.

The seasonal variations in the mineral content of the different parts as well as the whole plant of ryegrass and barley were studied. The data were calculated as N, P_2O_5 and K_2O for nitrogen, phosphorus and potassium respectively.

1. Nitrogen content. Data concerning the nitrogen concentration in the different parts of the ryegrass and barley plants were graphically illustrated in Figs 1 and 4.

The general trend of nitrogen percentage in the different parts, as well as the whole plant, in the different varieties studied decreased continuously with age. Such decrease noticed in the roots, stems and leaves in the first stages of growth might indicate a higher rate of dry matter production than nitrogen accumulation in these organs. Meanwhile, the decrease noticed at later stages of growth might be due to the translocation of this element to the spikes where it is mainly utilized by the developing seeds. The decrease observed in the spikes as well as the whole plant could be attributed to a higher dry matter production than nitrogen accumulation as they grew and developed.

Similar results were obtained with other crops belonging to the family *Gramineae* by ERDMAN (1929), FAGAN—WATKIN (1931), PHILLIPS *et al.* (1939), FUKIWARA—KOJI (1951), ABD EL-GAWAD (1959) and SHALABY (1959).

The increased values of nitrogen percentage of the stems noticed in the 6th and 7th samples of the Normal variety; 7th sample of Tetrone and 8th sample of Westerwolds might be due to a higher rate of translocation from other organs to the stems during these stages of growth. This might be a preparation for the immediate and large demands of flowering as well as seed development (LOEHWING 1940). Meanwhile, the increase noticed in the leaves at 86 days after sowing for the Tetrone variety and at the first sample of spikes for all ryegrass varieties might be due in part to a higher absorption rate of this element by the plant at these stages of growth. In this connection, SHALABY (1959) stated that there were two peaks with regard to the rate of nitrogen uptake by the rice plants. The first was at the time of tillering, whereas the second one was at the time of flower initiation. He suggested that plants had the most metabolic activity at these two stages. Moreover, MIKKELSON *et al.* (1958) found that rice absorbed a large amount of nitrogen during the tillering stage. Another period of high nitrogen requirement occurred at the heading stage.

Results showed also that the Tetrone variety exceeded barley and other ryegrass varieties in the nitrogen percentage of roots up to 116 days after sowing. This was true in the whole plant during the period from 86 to 116 days after sowing.

As for the total amount of nitrogen, data illustrated in Table 1 show that the general trend in barley roots, leaves and stems as well as the whole plant increased gradually with age to reach a maximum at 101 days after sowing then decreased till the end of the season. A noticeable increase was observed 71 days after sowing in the case of the leaves. The total nitrogen of the spikes showed a continuous increase as these organs grew and developed to reach a maximum at the end of the season. Results of similar character were obtained by **Abd El-Gawad** (1959) and **Shalaby** (1959) on rice.

The total nitrogen of ryegrass plants and their parts behaved in a similar way to that of barley though giving their maximum later in the season (146 or 161 days after sowing). An increase was noticed 101 days after sowing in the total nitrogen of the roots of the Tetrone variety. The total nitrogen of the spikes of the Normal variety followed the same trend as that of barley i.e. increased gradually to reach a maximum in the last sample. This increase might indicate a continuous accumulation of nitrogen at a higher rate than the dry matter production. The decrease noticed at the later stages of growth in the roots, stems and leaves might be a result of the translocation of nitrogen to the developing seeds. Meanwhile, the

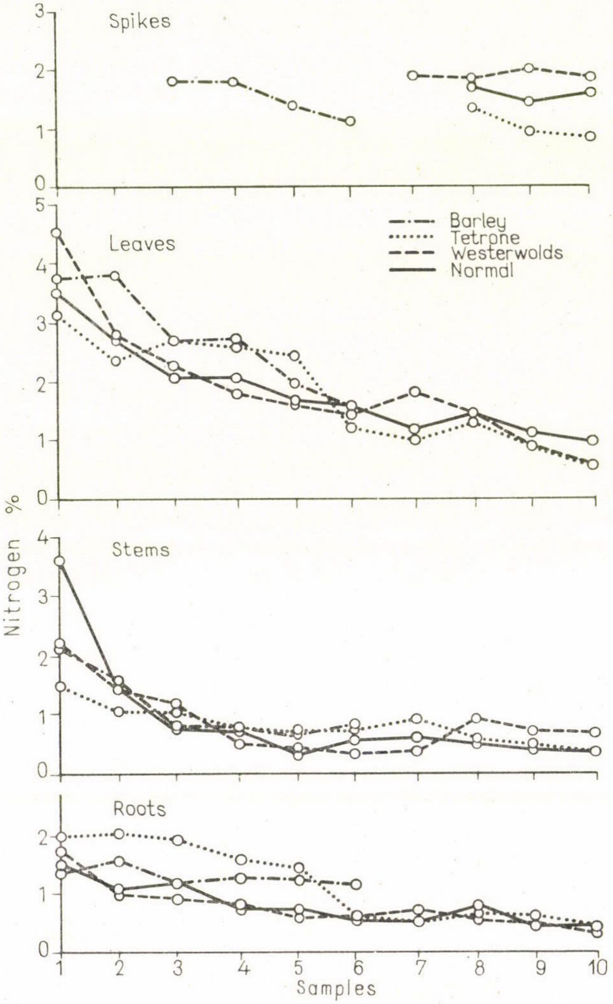

Fig. 1. Seasonal changes in nitrogen percentage of the different parts of barley and ryegrass plants

decrease noticed in the total nitrogen in the spikes of the Westerwolds and Tetrone varieties at the end of the season could mainly be due to the decrease in dry weight resulted from seed shattering noticed in these two varieties.

It could also be noticed that the barley plant gave its peak of total nitrogen two months prior to that of the ryegrass varieties. Moreover, this value in barley greatly exceeded that of the ryegrass varieties during the first stages of growth up to 131 days after sowing. Its maximum amount of nitrogen was 22.33, 44.30 and 85.95 mg/plant higher than those of the Westerwolds, Tetrone and Normal varieties respectively. Comparing the total nitrogen in the ryegrass

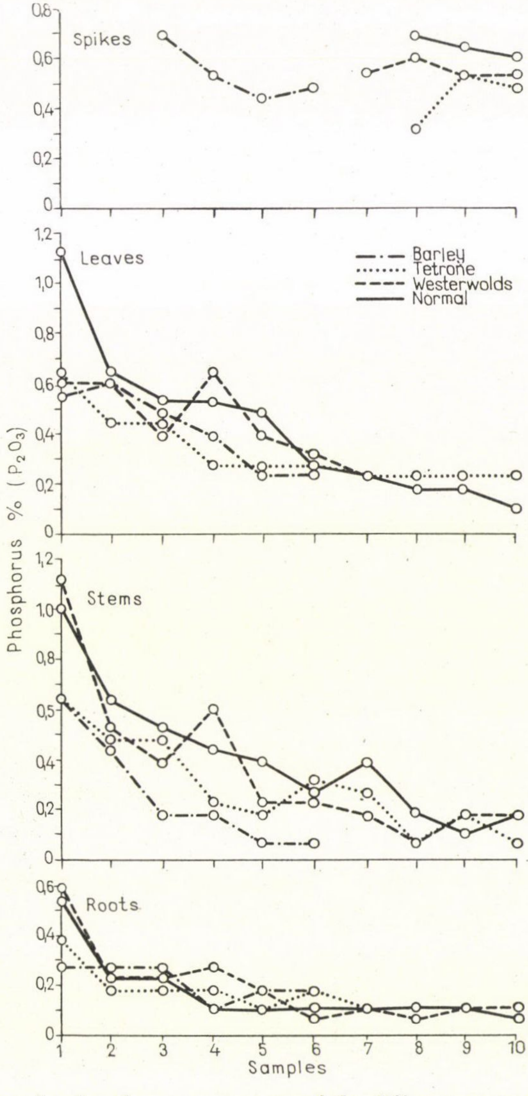

***Fig. 2.* Seasonal changes in phosphorus percentage of the different parts of barley and ryegrass plants**

varieties, generally Westerwolds showed higher values during the season followed by Tetrone, then the Normal variety.

From the above results, it could be indicated that the general trend of total nitrogen in plants of different varieties and their parts nearly followed that of the dry weight (El-Kadi *et al.* 1970) and might have some importance in using these varieties for forage production.

2. Phosphorus content. Data presented in Figs 2 and 4 show the seasonal changes in phosphorus percentage in the different organs of barley and ryegrass varieties. It is clear that the phosphorus percentage in the different organs as well as the whole plant in all the varieties studied generally began with initial high values then decreased continuously as the plants advanced in age till the end of the experiment. Similar results were reported by Fagan (1928), on Italian ryegrass, Ayers (1936), on sugar cane, Moxon *et al.* (1951), on prairie grasses and Shalaby (1959), on rice.

The decrease noticed in the percentage of phosphorus with the advancement in age could be mainly due to a higher rate of dry matter production than phosphorus accumulation.

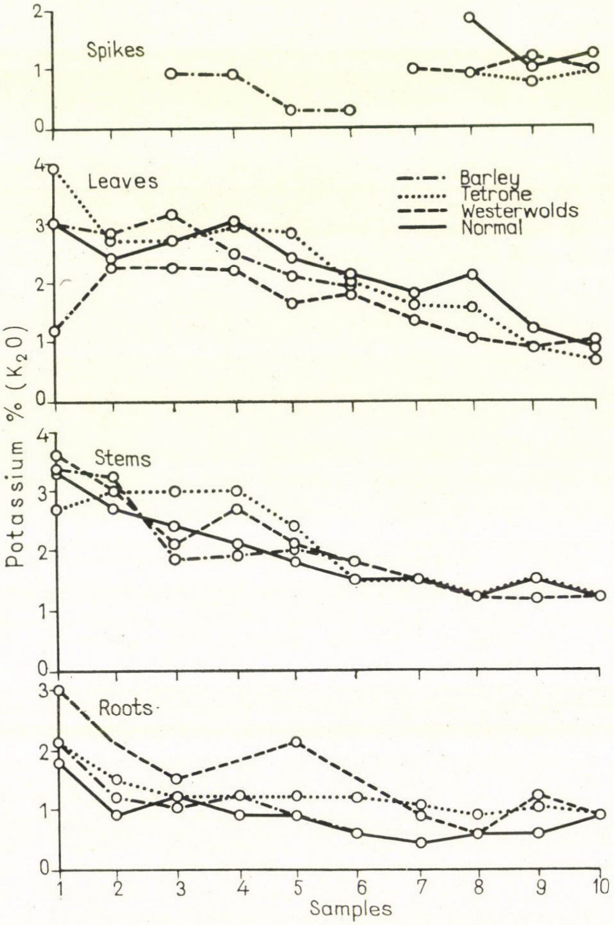

Fig. 3. Seasonal changes in potassium percentage of the different parts of barley and ryegrass plants

Meanwhile, such decrease in later stages in the roots, stems and leaves could be mainly due to the translocation of this element to the developing spikes. In this connection, SAYRE (1948), found that a movement of phosphorus occurred from the leaves of the corn plant into the grain as long as it was forming.

It is also clear from the data that barley stems have lower values than the other varieties throughout the whole experiment. Moreover, the phosphorus percentage of the Normal and Westerwolds varieties generally exceeded that of barley and Tetrone up to 116 days after sowing. This might bear some importance in using these varieties in forage production.

The phosphorus percentage of the whole plant of the Westerwolds variety showed an increase 101 days after sowing coinciding with a similar increase noticed in the different organs of the same plant. Such an increase might indicate an active period of phosphorus absorption at this stage of growth.

As for the total phosphorus (Table 2), the general trend of barley and ryegrass varieties nearly followed that of the total nitrogen and could be discussed similarly. A similar trend was

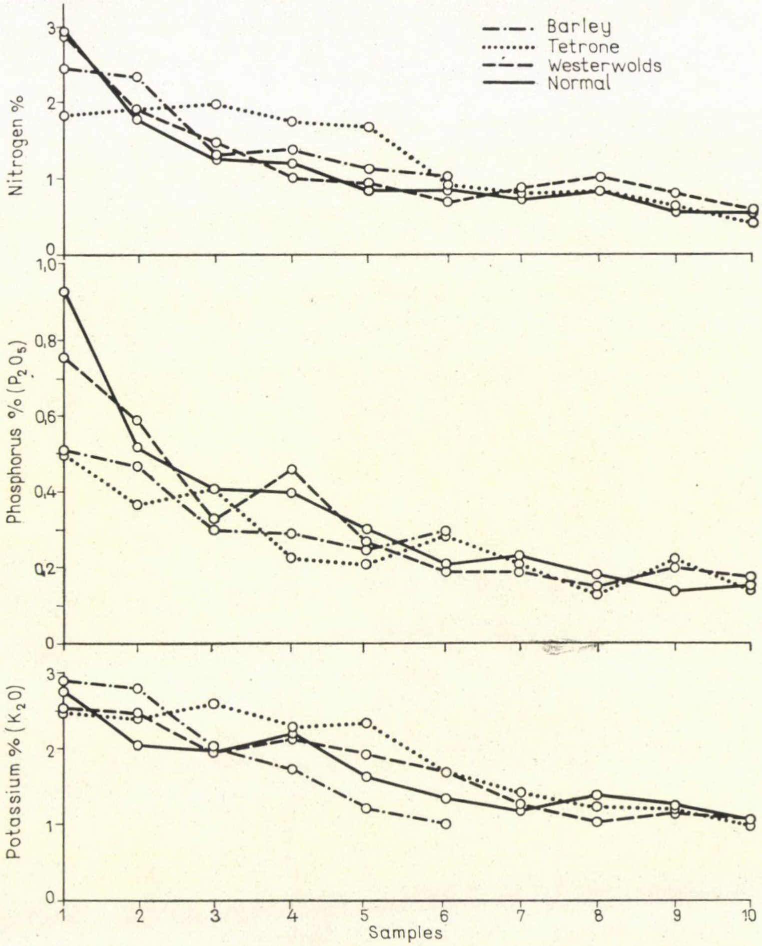

Fig. 4. **Seasonal changes in nitrogen phosphorus and potassium percentages of barley and ryegrass plants**

Table 1

Seasonal changes in total nitrogen (mg/plant) of the plant and its different parts of barley and ryegrass varieties

Samples	1	2	3	4	5	6	7	8	9	10
Days after sowing	55	71	86	101	116	131	146	161	167	191
Barley										
Roots	5.0	6.7	7.0	2.0	9.0	6.5	—	—	—	—
Stems	10.7	23.7	26.9	29.5	16.7	19.8	—	—	—	—
Leaves	17.5	41.6	34.7	42.2	15.6	15.5	—	—	—	—
Spikes	—	—	8.5	31.3	36.2	41.1	—	—	—	—
Whole plant	33.2	72.0	77.1	112.0	77.5	82.9	—	—	—	—
Tetrone										
Roots	1.6	1.2	2.5	7.1	4.0	1.7	6.2	7.5	3.8	4.1
Stems	0.9	0.5	2.8	2.0	2.2	5.8	11.9	23.3	9.9	6.5
Leaves	0.3	1.9	10.7	11.3	13.7	11.0	12.2	30.2	10.4	5.6
Spikes	—	—	—	—	—	—	—	6.7	3.7	2.3
Whole plant	2.8	3.6	16.0	20.4	19.9	18.5	30.3	67.7	27.8	18.5
Westerwolds										
Roots	1.0	0.5	3.2	3.6	4.6	9.0	15.5	7.1	4.9	5.2
Stems	1.3	0.9	3.2	1.1	2.3	4.6	11.9	44.2	18.2	12.7
Leaves	3.2	2.5	8.6	5.3	13.0	13.8	28.8	22.0	8.5	2.4
Spikes	—	—	—	—	—	—	7.0	16.4	10.4	5.1
Whole plant	5.5	3.9	15.0	10.0	19.9	27.4	63.2	89.7	42.0	25.4
Normal										
Roots	1.0	0.4	1.5	1.3	2.6	1.9	2.3	4.7	2.2	3.6
Stems	2.5	0.6	0.8	2.0	0.8	1.3	1.8	7.0	4.8	4.0
Leaves	3.5	1.3	1.6	6.1	4.3	4.0	3.8	13.2	5.0	5.5
Spikes	—	—	—	—	—	—	—	1.2	1.0	2.3
Whole plant	7.0	2.3	3.9	9.4	7.7	7.2	7.9	26.1	13.0	15.4

obtained by SHALABY (1959) on rice. In this connection, WAGNER (1933), working with the oat plant observed that after the absolute content of P_2O_5 had reached a maximum in the leaves, it was translocated from these organs to the fruiting ones.

It is clear from the results that the barley plant greatly exceeded the ryegrass varieties in the total amount of phosphorus during its life cycle recording its peak $1^1/_2$—2 months prior to that of the ryegrass varieties. This maximum value was 9.49, 12.62 and 17.99 mg/plant higher than that of the Westerwolds, Tetrone and Normal varieties respectively.

Comparing the total phosphorus in the ryegrass varieties, generally Westerwolds showed higher values during the season in the plant and its parts followed by Tetrone, then the Normal varieties.

3. Potassium content. The seasonal changes in the potassium percentage of the barley and ryegrass variety plants and their parts are shown in Figs 3 and 4. It could be noticed that

Table 2

Seasonal changes in total phosphorus (mg P_2O_5/plant) of the plant and its different parts of barley and ryegrass varieties

Samples	1	2	3	4	5	6	7	8	9	10
Barley										
Roots	1.0	1.2	1.7	0.8	1.3	1.0	—	—	—	—
Stems	3.3	6.7	6.1	7.0	1.8	1.7	—	—	—	—
Leaves	2.6	6.6	6.2	6.2	1.8	2.3	—	—	—	—
Spikes	—	—	3.3	9.4	11.7	18.1	—	—	—	—
Whole plant	6.9	14.5	17.3	23.4	16.6	23.1	—	—	—	—
Tetrone										
Roots	0.3	0.1	0.2	0.8	0.3	0.5	1.3	0.8	0.7	0.7
Stems	0.4	0.2	1.3	0.6	0.6	2.5	3.5	2.8	3.7	1.3
Leaves	0.1	0.4	1.8	1.2	1.5	2.5	2.8	5.5	2.8	2.4
Spikes	—	—	—	—	—	—	—	1.6	2.1	1.3
Whole plant	0.8	0.7	3.3	2.6	2.4	5.5	7.6	10.7	9.3	5.7
Westerwolds										
Roots	0.4	0.1	0.8	1.2	1.4	1.1	2.4	0.8	1.1	1.7
Stems	0.7	0.3	1.1	1.3	1.3	3.1	5.6	3.4	4.6	3.4
Leaves	0.4	0.6	1.5	1.9	3.2	3.1	3.7	2.8	1.7	0.5
Spikes	—	—	—	—	—	—	2.1	5.4	2.8	1.5
Whole plant	1.5	1.0	3.4	4.4	5.9	7.3	13.8	12.4	10.2	7.1
Normal										
Roots	0.4	0.1	0.3	0.2	0.4	0.4	0.5	0.7	0.5	0.6
Stems	0.7	0.3	0.6	1.3	1.0	0.7	1.2	2.5	1.3	2.1
Leaves	1.1	0.3	0.4	1.6	1.3	0.7	0.8	1.7	0.8	0.6
Spikes	—	—	—	—	—	—	—	0.5	0.5	0.9
Whole plant	2.2	0.7	1.3	3.1	2.7	1.8	2.5	5.4	3.1	4.2

all varieties generally showed a decrease throughout the season in the potassium percentage of the plant and its parts. Such a decrease could be mainly due to a high rate of dry matter production rather than potassium accumulation. Similar results were obtained by FAGAN (1928), PETRIC (1934), SAYRE (1948), NOMMIK (1955) and SHALABY (1959).

The initial high values obtained in the first samples of roots, stems and leaves as well as the whole plant of the different varieties might indicate a high rate of potassium absorption to meet the high demand of the developing cells at the first stages of growth. In this respect, BORDEN (1944) and LUNDEGARDH (1951) found that sugar cane and wheat take up potassium more rapidly during early stages of growth. It was suggested that the increase in potassium concentration early in the life of the plant may be attributed to the active metabolic stage.

It could also be noticed that the Westerwolds variety began with a low value of potassium percentage in the first sample of leaves, increased to reach its maximum value in the following two samples, then decreased till the end of the season.

Table 3

Seasonal changes in total potassium (mg K_2O/plant) of the plant and its different parts of barley and ryegrass varieties

Samples	1	2	3	4	5	6	7	8	9	10
Barley										
Roots	7.8	5.2	6.4	8.6	6.7	3.4	—	—	—	—
Stems	17.2	49.4	63.6	74.9	50.6	44.7	—	—	—	—
Leaves	14.2	31.7	40.7	39.3	17.0	19.3	—	—	—	—
Spikes	—	—	4.5	15.9	8.0	10.9	—	—	—	—
Whole plant	39.2	85.7	115.2	138.7	82.3	78.3	—	—	—	—
Tetrone										
Roots	1.7	0.9	1.6	5.5	3.4	3.4	12.1	10.2	6.7	8.5
Stems	1.6	1.5	8.1	7.8	7.5	11.9	19.5	47.8	31.3	21.8
Leaves	0.4	2.2	10.8	12.8	16.1	18.3	20.3	37.7	10.8	7.3
Spikes	—	—	—	—	—	—	—	4.5	3.0	2.5
Whole plant	3.7	4.6	20.5	26.1	27.0	33.6	51.9	100.2	51.8	40.1
Westerwolds										
Roots	1.8	1.1	5.4	8.3	16.0	22.6	19.6	7.2	11.8	13.5
Stems	2.2	1.8	5.9	6.0	11.6	24.4	47.3	58.1	30.6	23.0
Leaves	0.8	2.0	8.6	6.6	13.5	17.6	21.9	16.3	8.6	4.3
Spikes	—	—	—	—	—	—	3.7	8.1	6.0	2.7
Whole plant	4.8	4.9	19.9	20.9	41.1	64.6	92.5	89.7	57.0	43.5
Normal										
Roots	1.3	0.3	1.6	1.6	3.2	2.0	2.0	3.7	2.7	7.4
Stems	2.3	1.1	2.6	6.1	4.7	3.6	4.7	16.6	17.8	13.9
Leaves	3.0	1.2	2.2	9.0	6.3	5.6	6.0	19.8	5.6	5.2
Spikes	—	—	—	—	—	—	—	1.3	0.7	1.8
Whole plant	6.6	2.6	6.4	16.7	11.1	12.7	41.4	26.8	26.8	28.3

Comparing the differences between the studied varieties, it is clear that the potassium percentage of Westerwolds roots exceeded the other varieties during the first stages of growth up to 131 days after sowing. The leaves of the same variety showed nearly the lowest values throughout the whole experiment. On the other hand, the roots of the Normal variety gave lower magnitudes throughout the whole season. Although there were no clear differences between the stems of the varieties studied throughout the whole season, the Tetrone variety showed the highest while barley showed the lowest values during the period from 86 to 116 days after sowing. In this connection, WILSON (1889) analysed various grasses at different growth stages and found that the differences between the composition of the various grasses cut at the same stage are small.

Regarding the total amount of potassium in the plant and its component parts of the different varieties studied (Table 3), a trend nearly similar to that of the nitrogen or phosphorus was obtained and could be discussed similarly. Similar results were obtained by SHALABY (1959).

Comparing the differences between varieties, the roots of the Normal ryegrass variety recorded lower magnitudes of the total amount of potassium than the other varieties throughout the whole season. It has to be mentioned that the decrease noticed in the percentage and total potassium of the plants of the different varieties at later stages of growth might indicate a migration of this element back to the soil. A similar approach was reported by RICHARDSON *et al.* (1932), who stated that a loss of 27.8% of the total intake of potash by the herbage was sustained from all portions of the plant during the final phases of growth. They concluded that this loss occurred as a migration of potash from the plant to the soil due to the passive diffusion from the root system at a stage when physiological activity was declining.

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Prepared at the Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo: Department of Plant Production, Faculty of Agriculture, Azhar University.

A. RAAFAT, M. EL-KADI, S. H. EL-GHAYATY

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DIPTEROLOGICAL STUDIES IN SOME HUNGARIAN LARGE-SCALE PIG FARMS

While studying some dipterological questions of large-scale livestock farming we made investigations in three pig farms — at the Rózsás establishment of the Szarvas State Farm, in the pig combinate of the Szabadszentkirály Béke Co-operative Farm (Baranya County) and in the Fattening Farm of Nagytétény — in 1972.

Our work, and mainly the evaluation of the results were made extremely difficult by the fact that no similar investigations had so far been made in Hungary, and the foreign studies did not offer a sufficient basis for comparison. **Kühlhorn** (1961—68) carried on physical (temperature, humidity and CO_2 content) and dipterological investigations in German small farms over a long period. His work will not be criticized here, but it must be pointed out that he did not obtain any results that could be applied in large-scale livestock farming. As to the ecology of certain fly species transmitting diseases highly valuable works have been published (e.g. **Thomsen** 1938), but they can only help indirectly in solving the dipterological problems of concrete livestock units, since utilizable results can only be obtained by a simultaneous study of all fly species and the ecological conditions, on one hand, and the same fly species may have a different role in each farm, depending on the keeping conditions, on the other. Morphological studies performed by **Schumann** (1954, 1962) with coprophagous fly larvae also gave valuable and well applicable results to ecologists. From a certain point of view the work carried out by **Groth—Berndt** (1970) to find out the dispersion of house-flies in the stables with a more efficient chemical control in view is also important, but — as it will be pointed out subsequently — the destruction of imagines without the liquidation of breeding sites does not yield any result.

The greatest difficulty of similar ecological studies lies in the identification of species belonging to different fly families, since the specialists of the individual fly families — who are overtasked anyway — are not readily engaged in identifying an "ecological fly material" (i.e. a great number of flies collected or raised with the most detailed possible recording of ecological factors), although the precise identification of the species is the basis of all investigations. Owing to the superficial knowledge and misidentification of fly species and larvae many erroneous data have been published already, some of them even in such summarizing works as e.g MARTINI's book (1952). The most reliable way of obtaining correct results is when the fly specialists themselves start ecological investigations (MIHÁLYI 1965).

It is obvious even without any closer examination that the fly conditions of a pig-farm unit depend on the zootechnical facilities on one hand, and on the treatment of manure, on the other. The former primarily has a veterinary role while the latter — at least in the same measure — also public health implications. Prior to the discussion of the obtained results, the highly different keeping and manure handling conditions of the three farms will be outlined briefly.

The equipment of the Rózsás unit of the Szarvas State Farm (some 6500 animals; breeding farm and fattening farm) cannot be considered modern: extensive fly breeding may take place in the stables mainly on account of the palisades used as partition walls and troughs which do not meet the requirements of animal hygiene. On the other hand, the method developed in the Szarvas State Farm for handling the liquid manure (Fig. 1) seems to be optimal from a dipterological point of view. The pig-farm of the Béke Co-operative Farm of Szabadszentkirály (3500—4000 animals; breeding and fattening farm) is — from a zootechnical aspect — one of the most up-to-date farms, in the stables flies harmful to health have less opportunity to develop, and by finding the breeding places of the flies the stables can be made practically completely free of flies. The straw-bale filtering method (Fig. 2) used for handling manure derived from stables cleaned with water can hardly be criticized from a sanitary-dipterological point of view. There is no breeding unit in the Fattening Farm of Nagytétény but it serves for fattening an extremely large number of pigs (40—90,000). The out-of-date stables have been made free of flies by a careful chemical treatment, but in the runways large numbers of flies

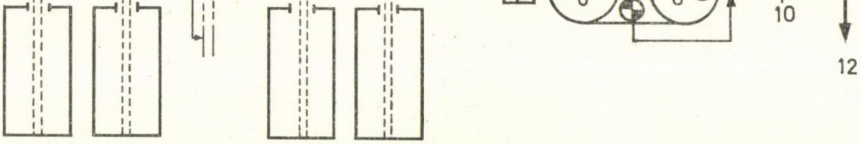

Fig. 1. Method of liquid manure handling developed by the State Farm of Szarvas (1 = Breeding farm; 2 = Sink holes; 3 = Fattening farm; 4 = Drain grating; 5 = Dilution water; 6 = Sink hole; 7 = Water reservoir; 8 = Stirring hole; 9 = Liquid manure storage basin; 10 = Irrigation; 11 = Sprinkling irrigation of field crops, 80%; 12 = Surface irrigation of poplars, 20%)

may develop. The mud-like manure is conveyed in trucks to the handling place (Fig. 3), where it is poured into a shallow basin. From here it is lifted into a mixer where it is mixed (rather unevenly) with peat, then loaded to waggons, or stored outdoors on the ground. In spite of the frequent chemical fly control, flies (mainly *Musca domestica* L.) develop on the manure ground in an almost unimaginable number.

We collected flies in various parts of each of the three farms, and from the stables — and in different phases of manure handling – took manure samples of 20—40 g dry matter content each, of which the flies were raised in a thermostat and identified — with the exception of some fly families represented by a low number and therefore insignificant. The results obtained are summarized in two tables.

Our results are analysed here first of all from a veterinary and public health point of view. First the fly conditions of the Szarvas Farm will be outlined on the basis of the tables.

In spite of the fact that the stables are cleaned with water-jet, owing to the inadequate equipment of the stables this does not give perfect results. At the points where the troughs are joined and in the gaps of the partition walls the manure and feed leftovers accumulate which renders the development of large numbers of flies — first of all the house-fly — possible. In

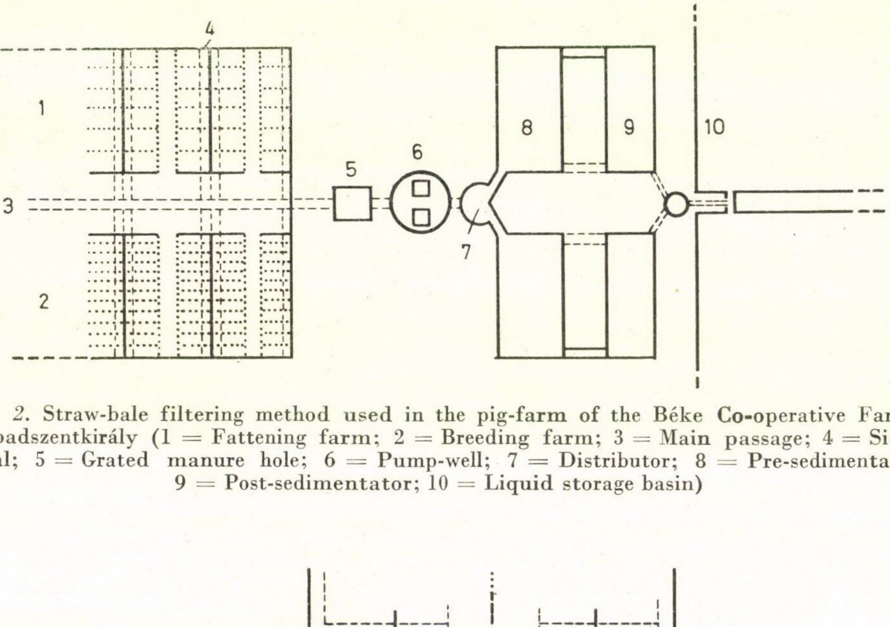

Fig. 2. Straw-bale filtering method used in the pig-farm of the Béke Co-operative Farm, Szabadszentkirály (1 = Fattening farm; 2 = Breeding farm; 3 = Main passage; 4 = Side-canal; 5 = Grated manure hole; 6 = Pump-well; 7 = Distributor; 8 = Pre-sedimentator 9 = Post-sedimentator; 10 = Liquid storage basin)

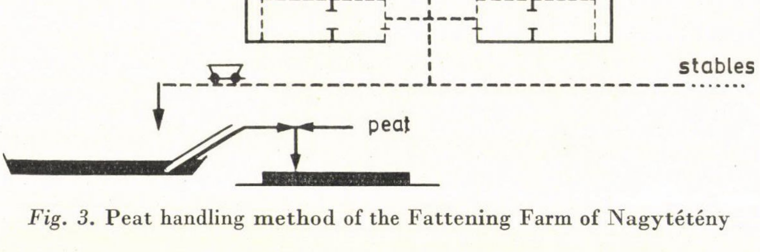

Fig. 3. Peat handling method of the Fattening Farm of Nagytétény

samples of about 20—30 g dry matter content taken from such places 123 houseflies developed. It is thus easy to understand that in the stables and feed-stores the number of flies dangerous to health is very high. Chemical control apparently does not solve the problem, only decreases its seriousness, while at the same time the situation cannot be changed with the present equipment. It must by all means be noted that owing to its small flying distance the house-fly is only able to transmit a potential infection from one stable to another. Infection from another farm cannot be introduced through flies, only by human transmission which can be excluded by various methods of sterilization. Flies on the embankment by the farm have gathered partly from the pig-farm, partly from the manure grounds (I/11), but in the nearby poplar grove (I/9) no house-fly can be found. On the basis of the data of investigations we can state that flies found in the stables develop only in the stables of the farm, and their vector role is restricted to the area of the farm.

In the pig-farm of Szarvas we had an opportunity to study two methods of manure handling. Namely, in the breeding farm the manure washed off by water-jet is carried into separate sunk basins (Fig. 1), from where it is conveyed by a snifting truck to the opening of the pipe-line. Over the sunk basins many drone flies could be seen, and many other harmless species caught (I/4) and raised (II/2) respectively. It could be seen even here that the liquid manure is not suitable for synanthropic flies to develop in. Among a total of more than 5000 flies caught in different parts of the manure grounds and hatched from manure samples not a single fly dangerous to either animal or man was found. Even the open drain grating and the material removed when it is cleaned are not suitable e.g. for house-flies to develop in. Manure accumulating in two or three days in the liquid manure storage basin does not exclude the development of flies, only that of disease vector species whose larvae do not survive in the high water content liquid manure. In comparison to other methods this one checks the development of other fly species too (see below). Since the material also arrives at the storage basin from the skin-holes of the breeding farm, and in summer the larvae of certain fly species may develop in any case in three days to a stage suitable for pupation, it was not surprising that in the supernatant of the storage basin fully developed larvae were found (*Themira putris* L.), and from the samples taken flies were raised on both occasions. The developed larvae are supposed to climb out of the basin and enter the pupa state in the surrounding soil. Samples taken on the two occasions corresponded to about 10 g dry matter each: 12 and 15 flies, respectively, developed from them which roughly corresponded to one fly per g dry matter. Thus in the storage basin of 270 m^3 cubic capacity receiving liquid manure with an average dry matter content of 7.5 g/litre, an amount of manure corresponding to 2025 kg dry matter accumulates in two or three days on an average: however, supernatant where flies may develop is formed only from 15 per cent of it. Therefore in the storage basin potentially hardly more than 300,000 tiny harmless flies can develop every 2—3 days in the summer period. This only appears to be a great number at first sight. Namely, according to our measurements and estimates, from an amount of pig manure corresponding to 1 kg dry matter theoretically 10,000 house-flies may develop, and 60 per cent of this value was also obtained in the present series of examinations ! Thus, this method provides a possibility that flies developing from the manure will be harmless and their numbers reduced almost by two orders of magnitude compared to those developing from the same amount of manure handled dry.

From the storage basin a part of the liquid manure — having been mixed — is used for the irrigation of medicinal plants at about 10 atmospheric pressure. These areas amount to 80 per cent of the total area irrigated with liquid manure. Larvae possibly carried there by the irrigation water are killed under such a high pressure, and in soils thus irrigated a relatively low number of coprophagous and "mud" flies could be caught (I/10). Another part of the liquid manure is used for the irrigation of poplars at about 1.5 atmospherical pressure, using deep furrow and stripe irrigation methods. Poplars are grown on 20 per cent of the total

Table 1

Collected flies

Species	Samples	Szarvas					
	T	1	2	3	4	5	6
Scatopse brevicornis Meig.	C	—	—	—	—	1	—
Scatopse notata L.	C	—	—	—	—	—	—
Cecidomyiidae indet.	X	—	—	—	—	—	—
Sciaridae indet.	C	—	—	—	—	1	—
Culicidae indet.	X	—	1	—	—	—	—
Chironomidae indet.	C ?	—	—	—	—	—	2
Ceratopogonidae indet.	X	—	—	—	—	—	—
Drapetis aenescens Wied.	C	—	—	—	—	—	2
Empididae indet.	—	—	—	—	—	—	—
Dolichopodidae indet.	X	—	—	—	—	—	—
Chloromyia formosa Scop.	C	—	—	—	—	—	2
Sphaerophoria scripta L.	?	—	—	—	—	—	—
Eristalomyia tenax L.	C	—	—	—	+	2	—
Lathyrophthalmus aeneus Scop.	C	—	—	—	1	—	—
Syritta pipiens L.	?	—	—	—	—	—	2
Phoridae indet.	C	—	1	3	—	—	3
Themira minor Halid.	I	—	—	—	—	—	—
Themira putris L.	C	—	1	—	37	4	56
Sepsis fulgens Hoffm.	C	—	—	—	1	—	71
Sepsis thoracica R.-D.	C	—	—	—	—	—	—
Sepsis violacea Meig.	C	—	—	—	2	—	—
Ulidia erythrophthalma Meig.	C	—	—	—	1	—	—
Opomyza florum Fabr.	X	—	—	—	—	—	—
Ephydridae indet.	I	—	—	—	—	—	—
Scatella stagnalis Fall.	I	—	—	—	—	—	—
Sphaerocera curvipes Latr.	C	—	—	—	1	—	13
Ischiolepta pusilla Fall.	C	—	—	—	—	—	—
I. vaporariorum Halid.	C	—	—	—	—	—	3
Coproica acutangula Zett.	C	—	—	—	—	—	1
Coproica digitata Duda	C	—	—	—	—	—	—
Coproica ferruginata Stenh.	C	—	—	—	19	—	350
Coproica hirticula Coll.	C	—	—	—	—	—	31
Coproica lugubris Halid.	C	—	—	—	—	—	3
Coproica vagans Halid.	C	—	2	—	39	9	354
Elachisoma aterrima Halid.	C	—	—	—	1	—	13
Elachisoma pilosa Duda	C	—	—	—	—	—	2

					Szabadszentkirály						Nagytétény
7	8	9	10	11	12	13	14	15	16	17	18
—	1	—	—	—	—	—	—	—	—	5	3
—	—	—	—	—	—	—	—	—	—	—	13
—	—	—	—	—	—	—	—	—	—	1	—
1	—	1	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	1	—	—	—	—
—	—	—	—	1	—	—	—	1	1	1	49
—	—	—	—	—	—	—	—	1	—	—	—
1	—	—	—	—	—	—	—	—	1	1	—
—	1	5	—	—	—	—	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	3
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	1	—	—	—
—	—	—	—	—	—	—	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	1	—	—	—	1	1	7	1
—	—	2	—	—	—	—	—	—	—	—	—
145	2099	330	9	98	—	—	—	4	103	44	—
2	18	87	—	2	—	—	—	9	2	8	—
—	—	1	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	2	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—
1	—	30	2	2	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—
1	—	2	—	—	—	—	—	—	—	—	1
1	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	3	1	—	—
1	—	—	—	—	—	—	—	—	—	—	—
7	6	—	—	—	—	—	—	80	270	8	67
—	1	20	—	—	—	—	—	19	—	2	35
—	—	—	—	—	—	—	—	—	—	—	—
1	112	544	1	—	—	—	—	23	6	3	796
2	2	31	—	—	—	—	—	5	3	25	3
2	—	—	—	—	—	—	—	—	—	—	1

Table 1 (Cont.)

Species	Samples	Szarvas					
	T	1	2	3	4	5	6
Trachyopella atoma Rond.	C	—	—	—	—	—	—
Trachyopella leucoptera Halid.	C	—	—	—	—	—	—
Trachyopella melania Halid.	C	—	—	—	1	—	—
Limosina bifrons Stenh.	C	—	1	—	—	—	27
Limosina crassimana Halid.	C	—	—	—	—	—	—
Limosina heteroneura Halid.	C	—	—	—	—	—	—
Limosina mirabilis Coll.	C	—	—	—	2	—	—
Limosina ochripes Meig.	C	—	—	—	—	—	—
Chaetopodella scutellaris Hal.	C	—	—	—	—	—	3
Leptocera (s. str.) *caenosa* Rd.	C	—	—	—	—	—	—
Leptocera curvinervis Stenh.	I	—	—	—	—	—	—
L. (Opacifrons) coxata Stenh.	I	—	—	—	—	—	—
L. (Rachispoda) breviceps Stenh.	I	—	—	—	—	—	—
L. (Rachispoda) modesta Duda	I	—	—	—	—	—	—
L. (Rachispoda) limosa Fall.	C, I	—	—	—	—	4	184
Scaptomyza pallida Zett.	X	—	—	—	—	—	—
Drosophila repleta Woll.	S	—	1	—	—	—	—
Agromyzidae indet.	X	—	—	—	1	—	—
Chloropidae indet.	X	—	—	1	3	—	—
Musca domestica L.	S	98	86	31	—	—	—
Stomoxys calcitrans L.	S	—	—	—	—	—	—
Hydrotaea glabricula Fall.	?	—	—	—	1	—	—
Fannia sp.	?	—	—	—	—	—	2
Paregle cinerella Fall.	S	—	—	—	1	—	—
Paregle radicum L.	S	—	—	—	—	—	—
Pegomyza sp.	X	—	—	—	—	—	—
Lucilia sericata Meig.	S	—	—	—	—	—	—
Sarcophagidae indet.	—	—	—	—	—	—	—
Tachinidae indet.	X	—	—	—	—	—	—
Total:		98	93	35	111	21	1124

T = ecological characteristics of species: C = species developing in manure or liquid manure; I = species living in mud; S = disease vector species; X = species not found in manure.

Flies caught in: 1 = one of the stables of the fattening farm on 8th June; 2 = one of the stables of the fattening farm on 11th August; 3 = the feed mixing store on 11th August; 3 = the feed mixing store on 11th August; 4 = above the individual sink-holes of the breeding farm on 11th August; 5 = at the opening of the pipe-line where the contents of sink-holes in the breeding farm are conveyed to by the snifting truck, on 8th June; 6 = on the material removed with the cleaning of the drain grating on 8th June; 7 = at the same place

					Szabadszentkirály						Nagy-tétény	
7	8	9	10	11	12	13	14	15	16	17	18	
—	—	—	—	—	—	—	—	2	2	1	5	
—	—	—	—	—	—	—	—	1	1	—	—	
1	—	—	—	—	—	—	—	—	—	1	—	
—	—	1	—	—	—	—	—	1	—	—	10	
—	—	22	—	—	—	—	—	—	—	—	—	
1	—	3	1	—	—	—	—	—	—	1	1	
—	—	—	—	—	—	—	—	—	—	1	3	
—	—	3	—	—	—	—	—	—	—	1	—	
—	—	3	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	4	
—	—	5	—	—	—	—	—	—	—	—	—	
—	—	3	1	—	—	—	—	—	—	—	—	
—	—	1	—	—	—	—	—	—	—	—	—	
—	—	1	—	—	—	—	—	—	—	—	—	
63	41	348	59	—	—	—	—	—	—	—	9	
—	—	—	—	—	—	—	—	—	—	—	3	
—	—	—	—	—	3	352	367	—	—	—	—	
—	—	1	—	3	—	—	—	—	—	—	—	
1	—	2	—	4	—	—	—	4	1	41	1	
—	—	—	—	2	39	—	—	1	—	—	108	
—	—	—	—	—	—	—	1	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	1	-	—	—	
—	—	—	—	—	—	—	—	—		—	1	
—	—	—	—	—	—	—	—	—	.	1	—	
—	—	—	—	2	—	—	—	—	1	—	—	
—	—	—	—	3	—	—	—	—	—	—	—	
—	—	—	—	3	—	—	—	—	—	—	—	
231	2281	1449	73	123	42	352	369	157	393	154	1120	8226

on 11th August; 8 = above the liquid manure storage basin on 8th June; 9 = above the moist soil of irrigated poplars on 8th June; 10 = above the soil of medicinal plants irrigated by sprinklers, on 11th August; 11 = with a net on the embankment beside of the pig-farm on 11th August; 12 = from the feed container placed in the main passage, on 30th July; 13 = at the same time from another feed container; 14 = one of the stables of the fattening farm; 15 = above the pre-sedimentator on 30th July; 16 = above the post-sedimentator on 30th July; 17 = at the edge of the liquid manure storage basin; 18 = flies caught in manure freshly delivered to the handling place and in that mixed with peat (22nd September).

Table 2

Reared flies

Species	Samples	Szarvas						
	T	1	2	3	4	5	6	7
Scatopse fuscipes Meig.	C	—	—	—	—	—	—	—
Psychoda alternata Say	C	—	101	—	4	—	—	—
Eristalomyia tenax L.	C	—	—	—	—	1	—	19
Lathyrophthalmus aeneus Scop.	C	—	1	—	—	—	—	—
Phoridae indet.	C	2	—	—	—	—	—	—
Themira putris L.	C	—	2	14	8	3	1	3
Sepsis fulgens Hoffm.	C	—	—	—	—	—	—	—
Sepsis violacea Meig.	C	—	—	—	—	—	—	—
Coproica ferruginata Stenh.	C	—	—	—	—	—	—	—
Coproica vagans Halid.	C	—	—	—	—	—	—	—
Limosina mirabilis Coll.	C	—	—	—	—	1	—	1
Limosina ochripes Meig.	C	—	—	—	—	—	—	—
L. (Rachispoda) limosa Fall.	C, I	—	—	—	—	—	—	6
Drosophila repleta Woll.	S	—	—	—	—	—	—	—
Desmometopa m-nigrum Zett.	C	—	—	—	—	—	—	—
Musca domestica L.	S	123	—	—	—	—	—	—
Muscina stabulans Fall.	S	1	—	—	—	—	—	—
Stomoxys calcitrans L.	S	—	—	—	—	—	—	—
Fannia leucosticta Meig.	S ?	—	—	—	—	—	—	—
Ophyra leucostoma Wied.	S	—	—	—	—	—	—	—
Paregle radicum L.	S	—	—	—	—	—	—	—
Lispe tentaculata Deg.	I	—	—	—	—	—	—	—
Bellieria melanura Meig.	S	—	—	—	—	—	—	—
Total:		126	104	14	12	5	1	29

Sample taken from: 1 = one of the stables of the fattening farm, from the space between the troughs and between the troughs and the palisades on 8th June; 2 = the sunk-holes of the breeding farm on 11th August; 3 = from the material removed from the drain grating when cleaned on 8th June; 4 = the liquid manure storage basin on 8th June; 5 = from the same place on 11th August; 6 = samples taken by filter from liquid manure applied to the poplar grove on the previous day (11th August); 7 = sample taken from the irrigated soil of the poplar grove on 8th June; 8 = manure collected in the stables of the fattening farm and breeding farm on 30th July; 9 = sample not spontaneously taken from manure left-over in the corner of stables and gaps of the concrete floor, and containing visible larvae

Szabadszentkirály						Nagytétény					
8	9!	10	11	12	13	14	15	16	17	18	19
—	719	—	—	—	2	—	—	—	1	—	—
—	3	—	—	4	1371	64	773	—	—	—	—
—	—	—	—	1	1	—	—	—	—	—	—
—	—	—	—	—	—	38	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	62	—	4	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	1	1
—	—	—	—	—	—	—	—	—	—	—	92
—	—	—	—	172	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	5
—	—	—	—	2	149	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—
—	—	—	—	—	—	—	—	—	—	—	—
1	—	7	2	—	—	—	—	—	—	—	—
—	—	53	—	—	—	—	—	—	—	—	—
—	6	4	—	—	—	—	—	—	n. ?	63	13
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	14	—	—
—	—	—	—	—	—	—	—	—	28	—	—
—	—	—	—	—	—	—	—	—	—	—	8
—	—	—	—	—	—	—	3	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	8
1	728 !	64	3	241	1523	106	776	0	44	64	127 $\sum_{1}^{19}=3968$

(see the text) (8th October); 10 = sample taken from walls and grating of side-canals in one of the farrowing pens (30th July); 11 = from the same place on 8th October; 12 = sample taken from the pre-sedimentator on 30th July; 13 = from the same place on 8th October; 14 = manure sample taken from the mud at the edge of the liquid manure storage basin on 30th July; 15 = from the same place on 8th October; 16 = from manure collected inside a stable on 22nd September; 17 = from the gaps of wooden fences dividing the runway, on 22nd September; 18 = sample taken from fresh manure delivered to the handling place (22nd September); 19 = from manure mixed with peat (22nd September).

irrigation area. In soils irrigated with this method a large number of flies develop (species characteristic of muds with high organic matter contents, II/7). From a sample taken from freshly applied liquid manure one fly developed, so it is possible though not sufficiently proved that from the storage basin fly larvae or eggs are carried through the pipeline to the soil of the irrigated poplars. It is sure that many coprophagous flies lay eggs on the irrigated soil, but these two ways of development of the fly population on the soil of the poplar grove are indifferent from our point of view, since the storage basin is not suitable either for synanthropic fly species to develop in. The method introduced by the Szarvas State Farm for handling liquid manure thus seems ideal from a sanitary-dipterological point of view, because it prevents the development of disease vector flies and reduces the smell of the large amount of manure to a minimum as well. This method of handling liquid manure may have disadvantages too from a veterinary point of view, owing to the wet environment and high air humidity, but these aspects are not included in our study.

In the pig-combinate of the Béke Co-operative Farm of Szabadszentkirály the stables are furnished with metal grating and modern equipment and washed with water-jet, so the manure remains on the surface for such a short time that flies cannot develop from it and even the possibility of eggs laid is low (II/8). Only a low amount of manure left occasionally on the concrete floor may serve a culture medium. (Sample II/9 was collected over a long period from such left-overs of manure in which larvae were found.) Thus from the manure seen in the stables a sufficient number of flies to explain the data of collections I/12—14 can by no means develop. Namely, beside stables almost completely free of flies there were stables where many *Drosophila repleta* were found above the automatic feeder, and considerable numbers of *Musca domestica* too on the feed tanks of the central passage. We soon realized that the breeding places of flies were in the side-canals of the gutter (Fig. 2) where between the grating and on the walls of the canals *Drosophila repleta* and *Musca domestica* could freely develop. This means that by a chemical treatment of the side-canals the pigfarm can be made free of flies, as in the different parts of the manure handling unit disease vector fly species occurring in the stables are found only exceptionally (I/15), and never develop there (II/12—15). The straw-bale filtering method of manure handling thus cannot be criticized for rendering possible the development of flies harmful to health. In certain parts of the manure handling unit large numbers of flies develop, but they are either the usual coprophagous species or those characteristic of high organic matter content muds, thus having no sanitary importance. For other reasons, however, the manure handling method of the State Farm of Szarvas is more advantageous. With the straw-bale filtering method the farms are deprived of a yield increase originating from irrigation with liquid manure, some farms cannot — or do not want to — utilize the manure accumulating in the sedimentators, so it represents a burden, such combinates must be established far from inhabited areas owing to the smell of the liquid manure storage basins.

Results obtained in the Fattening Farm of Nagytétény have confirmed our opinion that for hygienic reasons only some form of liquid manure handling can be the solution of manure handling in pig-farms. In manure samples taken from stables supplied with a low intensity of illumination and frequently treated with chemicals (II/16) no fly developed which is explained by the fact that in day-time the animals stay in the stables only for a short time so there is a relatively low amount of manure there, on the other hand, the inside arrangement of the stables renders a more efficient chemical fly control possible than e.g. in the Rózsás establishment (see before). On the other hand, in the gaps of the wooden fences dividing the runways manure samples could be taken full of the larvae of house-fly (still no house-fly developed from the larvae as the latter were destroyed by those of *Ophyra leucostoma* Wied. (cf. Mihályi 1965). Keeping the existing equipment, manure handling in the fattening farm is extremely bad and cannot be improved even from a dipterological point of view. With the above method of manure handling masses of house-flies and other synanthropic fly species develop even in October from both the fresh manure and that mixed with peat (II/18—19). No sufficient result is, and can be attained with the chemical control of larvae (Table 2), as they can be destroyed only with dissolved toxic materials, so the mud-like or solid manure can be poisoned only on the surface even if the whole surface of the manure can really be covered by toxic materials. Though poisoning reduces the number of larvae, there is a possibility of millions of house-flies developing here, and we suspect that the vast territory of fly breeding may have a role in the nearby inhabited area being infected with house-flies and other synanthropic species.

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Prepared at the Zoological Department of the Hungarian Natural History Museum, Budapest.

L. Papp

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AVAILABILITY OF P_2O_5 IN HIGHLY ALKALINE SOIL IN THE PRESENCE OF DIFFERENT CALCIUM SALTS

The addition of calcium salts to acid soils in judicious amounts increases their pH to a desired level and thus brings about a condition in them suitable for plant growth and development (HAUSENBUILLER 1963). This ion has a similar effect when added in the alkali soil.

The effect of calcium salts on the availability of P_2O_5 has not been much studied so far especially not in Indian soils. Recently, DE—ALI (1970) observed that the addition of calcium salts in general, increased the availability of P_2O_5 in slightly alkaline soil. Other Indian works available on the problem of the availability of soil phosphorus in the presence or absence of lime and liming materials were reviewed briefly by DE (1963). In this paper an attempt has been made to elucidate the effect of eight calcium salts viz., calcium carbonate, calcium oxide, calcium sulphate, calcium sulphide, calcium sulphite, calcium nitrate, calcium acetate and calcium citrate on the availability of soil P_2O_5.

A highly alkaline soil was collected from Soraon (Allahabad/U.P.) from a depth of 0—20 cm. The soil was completely dried, pulverized and passed through a 100-mesh sieve (British standard). The soil was then analysed chemically (DE 1965).

Physico-chemical composition of Soraon soil
(0 to 20 cm)

	%
HCl-insoluble	86.6
Fe_2O_3	3.0
Sesquioside	10.4
Total CaO	0.7
Total Na_2O	0.4
Total P_2O_5	0.21
Total organic carbon	0.015
Total nitrogen	0.0014
C/N ratio	10.7
Available P_2O_5	0.098
Total C.E.C.	18.8 m.e. per 100 g
Total exchangeable Ca^{++}	6.85 m.e. per 100 g
Total exchangeable Na^{+}	7.5 m.e. per 100 g
pH	10.6
Texture	Clay Loam

In order to observe the effect of different calcium salts on the availability of P_2O_5 in highly alkaline soil, the following two treatments were applied.

Treatment A: The soil was saturated three times with distilled water and dried after each of the first two saturations. After the third saturation with water, calcium salts equivalent to 1 m.e., 2 m.e., 4 m.e., 8 m.e., 16 m.e. and 20 m.e. were added per 100 g of soil. The experimental plates were then allowed to stand in one case for one and in the other for two months.

Treatment B: In this case, the soil was saturated three times with distilled water and subsequently dried after each saturation. After the third drying, the calcium salts were added in the fashion of treatment 'A' and kept for one and two months for reaction.

For each saturation, 55 ml of distilled water were required per 100 g of the experimental soil. For each set, a control was also run side by side.

After one and two months, the experimental plates containing the soils were analysed for available P_2O_5 colorimetrically (DATTA 1963).

The pH measurements were performed JACKSON (1958) by a Leeds—Northrup pH meter. The initial pH values of the treated soils after the addition of calcium salts were determined before whereas the final pH values were determined after the incubation of the treated soils.

A perusal of Tables 1 and 2 shows that the addition of different calcium salts in all the treated soils increases the availability of P_2O_5. A maximum availability of P_2O_5 was obtained by the addition of 8 m.e. calcium salts per 100 g soil. With the exception of calcium nitrate, calcium sulphite and calcium sulphate 16 m.e. calcium salts per 100 g soil gave the maximum availability of P_2O_5. Tables 1 and 2 show the effects of the different calcium salts with 8 m.e. concentration at which a maximum availability of P_2O_5 was obtained, with one and two month reaction times.

Table 1

Availability of P_2O_5 in highly alkaline (Soraon) soil by the addition of 8 m.e. of calcium salts per 100 g soil

Calcium salts	Treatment A						
	Av. P_2O_5 (p.c.) ($X \times 10^2$)		Increased Av. P_2O_5 ($X \times 10^2$) kg/ha		pH		
					Initial	Final	
	Time (months)		Time (months)			Time (months)	
	1	2	1	2	0	1	2
Calcium carbonate	13.6	15.2	40.7	86.9	10.50	10.7	10.9
Calcium oxide	14.1	14.1	70.2	67.8	10.6	10.9	11.0
Calcium sulphate	16.5*	15.9	52.6	99.7	10.0	9.5	9.7
Calcium sulphide	14.5	15.9	51.5	99.7	10.3	10.1	10.1
Calcium sulphite	16.8*	20.9	87.3	183.7	9.7	9.3	9.5
Calcium nitrate	20.9*	22.8	105.1	215.6	8.9	8.7	8.6
Calcium acetate	16.3	19.6	72.6	143.5	10.2	9.9	10.1
Calcium citrate	16.6	19.5	75.0	143.5	10.2	9.8	9.0
Control	9.9	10.0	—	—	10.5	10.5	10.5
Fischer's 't' value at 5% level of significance**	8.2	5.5					

* Available P_2O_5 content was determined from the treatment at 16 m.e. calcium salts per 100 g soil.

** The calculated 't' value for 7 d.f. at 5% level of significance = 2.365.

In Tables 1 and 2, it is evident that the availability increases in all cases by the addition of all the eight calcium salts in composition to the control. This measure is found to be greater in the case of the experimental plates kept for two months in treatment A then the one month plates of treatment B. This is obviously due to the fact that there is a critical moisture requirement which was not present in the case of treatment B for plates kept for two months and in the case of treatment A for plates kept for 1 month. In general, it is interesting to note that where there is decrease in the resultant pH with the addition of calcium salts to the strongly alkaline soil, there is an increase in the availability of P_2O_5 (RAYCHAUDHARI—LANDEY 1960). The increase is less when such a pH change is low as in the cases of calcium carbonate and calcium oxide (Tables 1 and 2).

It is interesting to note further that the values of available P_2O_5 in comparison to the control due to the addition of different calcium salts (Tables 1 and 2) are found to be significant at 5% level of significance from Fisher's 't' table.

The orders of effectivity of the eight calcium salts in relation to the availability of P_2O_5 in strongly alkaline soil for both the treatments under one and two months time of experimentation were observed to be the following;

Treatment A: $Ca(NO_3)_2 > CaSO_3 >$ Ca-Citrate $> Ca(CH_3COO)_2 > CaSO_4 > CaS > CaO >$
$> CaCO_3$

Table 2

Availability of P_2O_5 in highly alkaline (Soraon) soil by the addition of 8 m.e. of calcium salts per 100 g soil

Calcium salts	Treatment B						
	Av. P_2O_5 (%) ($X \times 10^2$)		Increased Av. P_2O_5 ($X \times 10^2$) (kg/ha)		pH		
					Initial	Final	
	Time (months)		Time (months)			Time (months)	
	1	2	1	2	0	1	2
Calcium carbonate	12.9	12.5	48.3	41.6	10.6	10.8	11.0
Calcium oxide	14.1	13.1	67.5	51.2	10.7	11.0	11.0
Calcium sulphate	17.3*	15.9	121.9	99.1	10.0	19.7	9.7
Calcium sulphide	14.8	14.4	80.3	96.0	10.2	10.1	10.0
Calcium sulphite	22.2*	21.9	204.1	198.1	10.0	9.2	9.8
Calcium nitrate	22.4*	22.2	208.0	204.5	9.3	8.8	8.8
Calcium acetate	18.6	17.8	144.2	114.2	10.3	10.2	10.2
Calcium citrate	19.9	19.0	155.3	150.2	10.2	10.0	10.1
Control	10.0	10.1	—	—	10.5	10.5	10.5
Fischer's 't' value at 5% level of significance**	4.3	3.8	—	—	—	—	—

* Available P_2O_5 content was determined from the treatment at 16 m.e. calcium salts per 100 g soil.

** The calculated 't' value for 7 d.f. at 5% level of significance = 2.365.

From the above orders, it may be observed that the addition of calcium nitrate gives the best maximum availability of P_2O_5 in both the treatments both when kept for one month and two months. The addition of calcium carbonate gave the lowest availability of P_2O_5 in both the treatments (Tr. A and B) under both reaction times (one and two months). Further, it may be observed that calcium salts with organic anions like acetate and citrate generally hold tolerable positions in the above efficiency orders.

The greater efficiency of calcium nitrate (Grunes 1959, Kanwar 1964) is due not only to its contribution to the reduction of soil pH but also to the readily available source of calcium in the strongly alkaline soil and to the good source of nitrogen so much required by the microorganisms responsible for partly decomposing the organo phosphorus compounds of the soil (Black 1957) resulting in an increased availability of P_2O_5. It is generally observed that the decomposition of phosphorus bearing organic matter or the growth of phosphate loving organisms are enhanced in the presence of nitrate (Waksman 1952) and acetate ions, the latter being more useful in the oxidation of carbohydrate and animal tissues (Frear 1950). This decomposition proceeds under better aeration which condition is available on the aggregation of the soil particles as a result of replacement of Na^+ ions by Ca^{++} ions in the soil exchange complex.

Incidentally, it may be mentioned here that Jenny—Martin—Vlamis (1960) showed in their experiments that the production of nitric acid by the addition of calcium nitrate in strongly alkaline soil increases the available form of soil phosphorus to an extent as great as did the application of about 175 lbs of fertilizer phosphorus per acre and with sulphuric acid which might have generally been produced with sulphite and sulphide salts of calcium increase the inter-action of soil phosphorus to an extent equivalent to 350 lbs of fertilizer phosphorus per acre (Gardner—Kelley 1940).

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Prepared at the Department of Chemistry, University of Allahabad, Allahabad.

S. K. De, S. Ali, D. Palit

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EFFECT OF DIFFERENT LEVELS OF IRON, PROTEIN AND CALCIUM ON GROWING CHICKS FED 25 PER CENT DECORTICATED COTTON SEED CAKE IN THEIR RATIONS

One of the most vital factors that limit poultry populations in their potential development is the lack of good quality protein feeds. Cottonseed meal is the most important protein concentrate in Egypt due to its volume of production and low price. It is usually the protein source of choice for ruminant animals. Improvements in cottonseed meal have to take place to make it possible to greatly increase the amounts of it used in poultry rations.

The widespread use of cottonseed meal in poultry rations depends upon the inherent quality of the protein and the amount of damage which has taken place during processing. Heat is the principal variable relating to damage. Heat is not the only factor in damage, however, chemical reactions of the protein during processing with other constituents such as pigments, oil and carbohydrate could influence the amount of protein damage incurred.

The significance of the existing gossypol-mineral interrelationships was recognized more than 50 years ago (ULLIREY 1966). Iron salts are quite effective in the detoxification of gossypol when added to the feed ration of nonruminant animals (ALLEN *et al.* 1966, CABELL—STEVENSON 1966, LYMAN 1966). Several workers reported that high levels of calcium in chick ration permitted normal growth rates when relatively high levels of gossypol were present.

The combination of iron and calcium had a synergistic effect in counteracting the effect of gossypol on the growing chicks (BRESSANI *et al.* 1966).

Therefore this work was undertaken to minimize the harmful effect of feeding high levels of decorticated cottonseed cake by supplementing the rations of the growing chicks with various levels of iron, calcium and protein.

For the present investigation, a hundred and eight Fayomi chicks, nine weeks old, were used. The birds were clinically healthy. At the start of the experiment each chick was weighed and marked with a numbered wing band, then the chicks were divided into nine groups (12 chicks each). The birds were weighed weekly during the experiment early in the morning prior to feeding, before each week and at the end of the experiment.

Rations offered to the nine groups are shown in Table 1. The values of the Digestible Protein (D.P.) as well as the Total Digestible Nutrients (T.D.N.) of the ingredients were taken after TITUS (1961) and ABOU-RAYA (1967). The rations were balanced in T.D.N., minerals (except iron and calcium) and vitamins.

Feed and water were given ad-libitum and a weekly record of feed consumption was kept.

Table 1

Composition of rations fed to various groups

Group Ingredient	I	II	III	IV	V	VI	VII	VIII	Control
Decorticated Cotton seed cake %	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	—
Wheat %	18.75	18.75	17.50	17.50	18.75	18.75	17.50	17.50	17.50
Horse bean %	3.75	3.75	3.50	3.50	3.75	3.75	3.50	3.50	12.50
Bran %	15.00	15.00	14.00	14.00	15.00	15.00	14.00	14.00	20.00
Maize %	30.00	31.50	27.80	29.30	30.00	31.50	27.80	29.30	42.00
Blood meal %	2.25	2.25	4.60	4.60	2.25	2.25	4.60	4.60	3.00
Meat meal %	2.25	2.25	4.60	4.60	2.25	2.25	4.60	4.60	3.00
Calcium carbonate%	2.50	1.00	2.50	1.00	2.50	1.00	2.50	1.00	1.00
Sodium chloride %	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
$FeSO_4$ ppm	100	100	100	100	400	400	400	400	40

The different levels of iron in a form of ferrous sulphate (RANDS 1966), calcium and protein added to rations of various groups separately or in combination were as follows:

Group	1	2	3	4	5	6	7	8	9 control
Calcium, %	2.5	1.0	2.5	1.0	2.5	1.0	2.5	1.0	1.0
Protein, %	20		24		20		24		15
Iron, ppm	100				400				40

The spectrophotometric determination of total gossypol in cottonseed meal was carried out in this work.

In order to evaluate the differences obtained among groups, the analysis of variance and Least Significant Differences (LSD) were carried out according to SNEDECOR (1968).

Data in Table 2 show a slight variation between groups in the average feed consumption per head during different periods. The lowest amount of feed consumed per head was found in Group VI, while the highest was consumed in Group VIII. Although the chick in the control group consumed the highest amount of feed in the first period, the total amount of feed consumed by the chick of such a group was moderate.

The average weekly gain percent (Table 3) in the first period was the highest in Group VI, while the lowest in Group II. At the last period, the highest gain per cent was obtained by Group V. Chicks of the control group had a normal gain percent.

It is evident from the results obtained in Table 4 and Figure 1, that the average gain per cent of chicks treated with the low level of iron was lower than that of those receiving the higher level during different periods (except the second period). Differences between the two treatments were highly significant. Results of the two other levels (protein and calcium) followed the same pattern already found with iron, yet the difference between the high and low level in each treatment was less.

Table 2

Average feed consumed by chicks of various groups during different periods

Group \ Periods in weeks	1	2	3	4	5	6	7	8	Total
	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
I	378	348	417	476	500	515	536	553	3723
II	360	368	440	496	500	537	532	553	3786
III	377	401	451	472	488	510	505	581	3785
VI	367	380	430	478	500	531	539	553	3848
V	360	386	430	479	484	477	532	579	3727
VI	383	368	445	473	480	507	487	527	3670
VII	352	362	423	488	500	518	554	533	3730
VIII	346	382	460	496	520	522	597	617	3940
Control	385	396	449	473	482	489	521	565	3763

Table 3

Average gain per cent of various groups during different periods

Group \ Periods in weeks	1	2	3	4	5	6	7	8
I	17	36	51	64	78	95	109	118
II	10	31	47	60	73	86	109	119
III	16	42	64	80	103	117	130	147
IV	17	38	52	78	92	115	139	145
V	19	39	64	82	105	127	145	155
VI	22	36	68	80	103	120	141	149
VII	16	35	59	78	94	119	136	149
VIII	15	35	57	72	90	110	130	142
Control	20	39	57	78	93	113	125	136

Table 4

Average gain per cent of chicks in various groups fed high or low levels of iron protein or calcium during different periods (each level extracted from data of four groups)

Treatments \ Period		1	2	3	4	5	6	7	8
Iron	100 ppm	15	37	54	71	87	103	122	132*
	400 ppm	18	36	62	78	98	119	138	149
Protein	20%	17	34	58	72	90	107	126	135
	24%	16	38	58	77	95	115	134	146
Calcium	1.0%	16	35	56	73	90	108	130	139
	2.5%	17	38	60	76	95	115	130	142

* Highly significant.
LSD for iron treatment was:

11.7	0.05
15.4	0.01

The high level of iron gave a higher gain per cent than not only the low level of iron, but also the high protein and calcium levels. On the other hand the lowest gain per cent was obtained with a low iron level. Such gain per cent was lower than even the low level of protein or calcium. While the low level of calcium was better than the low levels of iron or protein.

Data in Table 5 present the average body weight of chicks reared on high or low levels of iron, protein and calcium. Such results were in accordance with those obtained previously concerning the gain per cent of corresponding levels.

The average total gain (Table 6) ranged between 467 gms (Group I) and 576 gms (Group III) while the total gain of the control group was 515 gms. The higher amount of feed consumed

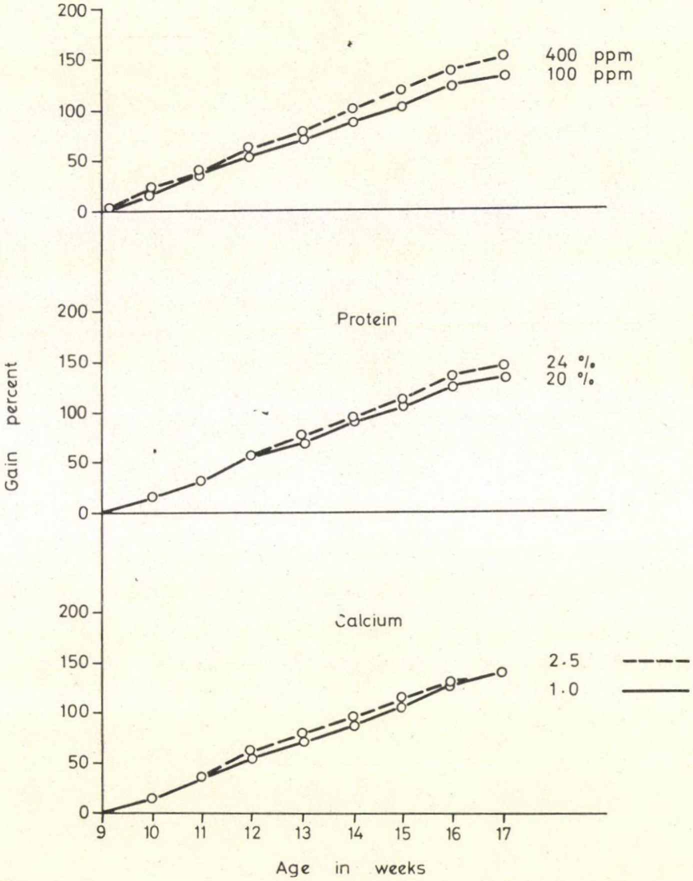

Fig. 1. Relationship between gain percentage and age of chicks receiving 25% decorticated cottonseed cake in their ration with different levels of iron, protein and calcium

during the whole experimental period was found in Group VIII, while the lowest amount was consumed by Group VI. As feed conversion is the resultant of feed consumption and the body weight gain of the chick, the lowest feed conversion was found in Group II (received the three low levels), while Group VII (received the three high levels) had a feed conversion of good order.

The feed conversion of groups receiving the high levels of iron, protein and calcium separately was better than that of those receiving the low levels (Table 7). It is evident from these results that the higher level of iron produced the best feed conversion, while the worst feed conversion was obtained by groups receiving the low level of iron. The difference between the two calcium levels was the lowest, while the highest difference was found between the two iron levels.

Analysis of variance of the data (Table 8) showed that differences between treatments were significant. Although the differences between the two levels of iron were highly significant, neither the differences between the two levels of protein nor the two levels of calcium were insignificant.

Table 5

Average body weight of chicks reared on various levels of iron, protein or calcium during different periods (each level extracted from the data of four groups)

Treatment	Periods in weeks	Initial weight	1	2	3	4	5	6	7	8
			gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Iron	100 ppm	387	445	530	595	660	722	786	856	899
	400 ppm	368	433	501	597	655	728	806	874	914
Protein	20%	377	439	511	592	645	712	777	847	883
	24%	378	438	520	599	670	738	814	883	929
Calcium	2.5%	379	443	522	606	666	738	811	865	915
	1.0%	376	435	508	585	649	712	780	862	897
Control		379	455	528	604	675	734	808	853	894

Table 6

Average total gain, feed consumption and feed conversion of different groups during the whole experimental periods

Group	Average total gain	Average total feed consumed	Average feed conversion
	gms.	gms.	gms.
I	467	3723	7.97
II	473	3786	8.00
III	576	3785	6.57
IV	529	3848	7.27
V	544	3727	6.85
VI	541	3670	6.78
VII	560	3730	6.66
VIII	538	3940	7.32
Control	515	3763	7.30

The Least Significant Difference (Table 9) between each of the two groups showed a highly significant difference between the high and low levels which involved it in the iron.

From the present results of this investigation, good evidence could be obtained concerning the positive effect of a higher level of iron on growth and feed conversion when the diet of the growing chicks contained 25% decorticated cottonseed cake. Addition of iron at a high level permitted the chick to grow better than the normal rates received by the control chick, even though 1750 ppm total gossypol was present in the ration. Such results indicated that iron when used in sufficient concentration, was effective in protecting chicks from the

Table 7

Average feed conversion of chicks fed high or low levels of iron, protein or calcium during the whole experimental period

Treatment / Level	Iron	Protein	Calcium	Average
High	6.90	6.98	7.01	6.96
Low	7.45	7.37	7.34	7.38
Control				7.30

Table 8

Analysis of variance in gain per cent of chicks in various groups studied in different periods of growth during the experimental period

S.V.	d.F.	S.S.	M.S.	F	Tabulated F		
					0.05	0.01	
Total	85	89,121	—	—	—	—	—
Treatments	7	14,566	2081	2.71	2.12	2.87	Significant
Iron	1	6,647	6647	8.64	3.96	6.96	Highly sig.
Protein	1	2,105	2105	2.74	3.96	6.96	Insig.
Calcium	1	848	848	1.10	3.96	6.96	Insig.
Inter. Iron × Pr.	1	2,053	2053	2.67	3.96	6.96	Insig.
Inter. Iron × Ca	1	2,866	2866	3.73	3.96	6.96	Insig.
Inter. Pr. × Ca	1	60	60	0.08	3.96	6.96	Insig.
Error	78	59,976	769	—	—	—	—

harmful effects of cottonseed meal. Several workers (Hopkins—Chilson 1966) came to the same conclusion and stated that iron was effective in protecting the chicks from the toxic effect of cottonseed meal.

Conflicting results were found when a low level of iron was used, i.e. low level of iron had no effect on the toxic effect of cottonseed meal. Therefore it is necessary for gossypol detoxification, a high level of iron must be present in such a ration. In that connection Smith—Clawson (1966) added ferrous sulphate to diets containing 244—400 ppm of free gossypol. A ratio of 0.5 : 1 iron to gossypol gave partial detoxification and a 1 : 1 ratio gave complete detoxification.

According to Jonassen's (1966) explanation iron protected chicks from the toxic effect of cottonseed meal due to the fact that the gossypol moiety interacted with a ferrous ion, the complex consisted of one Fe (II) ion and one gossypol ion to give the general formula Fe (II) gossypolate. Other authors believed that gossypol and iron formed an insoluble complex preventing the gossypol from being absorbed from the gastro-intestinal tract.

Moreover, it could be concluded that it is probable that more consistent results would be obtained if more iron were used in such a diet. In this aspect Altschul (1966) reported that there are problems in the relationship between the right quantity of iron and the quantity of gossypol.

Table 9

Least Significant Difference (LSD) among groups

Treatments		Per cent gain		Difference	LSD		Significant
a	b	a	b		0.05	0.01	
1	2	118	119	1	23.2	30.5	Insig.
1	3	118	147	29	23.8	31.3	Sig.
1	4	118	145	27	23.2	30.5	Sig.
1	5	118	155	37	23.2	30.5	Highly sig.
1	6	118	149	31	23.2	30.5	Highly sig.
1	7	118	149	31	23.2	30.5	Highly sig.
1	8	118	142	24	23.8	31.3	Sig.
2	3	119	147	28	23.8	31.3	Sig.
2	4	119	145	26	23.2	30.5	Sig.
2	5	119	155	36	23.2	30.5	Highly sig.
2	6	119	149	30	23.2	30.5	Sig.
2	7	119	149	30	23.2	30.5	Sig.
2	8	119	142	23	23.8	31.3	Critical
3	4	147	145	2	23.8	31.3	Insig.
3	5	147	155	8	23.8	31.3	Insig.
3	6	147	149	2	23.8	31.3	Insig.
3	7	147	149	2	23.8	31.3	Insig.
3	8	147	142	5	24.3	32.0	Insig.
4	5	145	155	10	23.2	30.5	Insig.
4	6	145	149	4	23.2	30.5	Insig.
4	7	145	149	4	23.2	30.5	Insig.
4	8	145	142	3	23.8	31.3	Insig.
5	6	155	149	6	23.2	30.5	Insig.
5	7	155	149	6	23.2	30.5	Insig.
5	8	155	142	13	23.8	31.3	Insig.
6	7	149	149	0	23.2	30.5	Insig.
6	8	149	142	7	23.8	31.3	Insig.
7	8	149	142	7	23.8	31.3	Insig.

The data obtained in the present study is of considerable practical interest. There was an improvement in body weight gain and feed conversion when a higher level of calcium was present. This finding is in agreement with that recommended by many investigators. On the contrary SMITH (1966) indicated that calcium did not protect pigs from mortality due to gossypol.

Although the high level of calcium protected chicks from the harmful effect attributed to gossypol, the protection was not so effective as with the high level of iron. In other words, separately added calcium and iron ration containing 25% cottonseed meal increased the final body weight, for which iron was better than calcium, such results might be due to the fact that the calcium-gossypol complex is not very stable and in the dynamic equilibrium existing in the digestion tract appreciable quantities of unbounded gossypol are absorbed (Rands 1966). Consideration must also be given to other components of the diet, such as phosphorus to balance the Ca/P ration and to micronutrients, such as Zn, since their utilization is affected by high intakes of calcium (Bressani *et al.* 1966).

The marked increase in the growth of chicks fed high levels of protein, was consequently accompanied by an improvement in feed conversion. Such results indicated that a high level of protein from other sources compensated the protein damage occurring during the processing of oil extraction from cottonseed, due to heat and chemical reactions. The cottonseed meal supplied 38% Ca from the total digestible protein in the ration. Several workers, among others Lyman (1966), recommended protein in high levels.

In that connection, it was reported (Phelps 1966) that commercial cottonseed meals seem to perform better in rations for broilers when supplemented with L-lysine, the next most limiting amino acids in cottonseed meals appeared to be methionine and one or all of the threonine, isoleucine and leucine group. However, Altschul (1966) reported that there are problems involving the relationship to the quality and amount of protein and the relationship to other metals such as calcium and to such as its effect on vitamin E.

It is evident from these results that iron was a more important additive in detoxifying the gossypol than the two other additives.

From the previous data, it could be concluded that separately added iron, calcium and protein to growing chicks rations increased the final body weight and improved feed conversion, for which iron was better than protein and calcium.

Combinations of three high levels of iron, protein and calcium has a synergistic effect in counteracting the effect of gossypol. Better results were obtained when such additives were in combination than when added to the rations separately. Similar results were recorded by Bressani *et al.* (1966).

In a previous study it had been recorded that the optimum level of decorticated cottonseed cake that could be fed to chicks must not exceed 15%, a drop in both gain and feed conversion occurred when further cottonseed cake was administered to the ration.

In this work as rations contained 25% decorticated cottonseed cake, when added with high levels of iron, calcium and protein better results than the control were obtained in both body weight gain and feed conversion.

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Prepared at the Department of Animal Production, Faculty of Agriculture, Assuit University, Cairo.

A. M. Darwish

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QUANTITATIVE MICROSCOPY

A microscope magnifies things optically, which can be detected by the naked eye only vaguely or not at all. The human eye is able to see shape and colour. This does not change with microscopical observation. The question comes up if a microscopical image is capable of giving quantitative data. For a long time there have been auxiliary means of performing quantitative determinations in certain specimens: object and eyepiece micrometers to measure lengths, integration plates to determine the particle size distribution and specimen slides divided into uniform areas to count and differentiate specimen particles. It is evident from these few examples that in this way physical dimensions or population numbers are measured. The chemist, however, usually has a different interpretation of quantitative determination: the measurement of the amount of a substance, the concentration of one component in a mixture, expressed in weight per unit volume. Some of the above mentioned microscopical measurements can be used under certain conditions, to measure quantitatively in this respect. A simplified example is the determination of the dispersed substance in an emulsion. One needs to count the number and measure the diameters of the sphere-shaped particles in a given volume under the microscope. The total amount of the emulsified substance can be computed. The measurements can be made visually or with at TV-microscope (e.g. the OPTON Micro-Videomat). However, the essential field of quantitative microscopy is the photometry of a microscopical specimen.

Photometry

In the proper sense photometry is the measurement of a lightflux per unit time. A trivial example is the measurement of the exposure time in photography. The readings from a photometer scale are very seldom the absolute amounts of lightflux e.g. photons per second. Normally the scales are modified to fit the application. Quantitative measurements usually require the comparison of two different lightfluxes: standard and sample are compared. For direct measurements of radiant bodies (e.g. in fluorometry) the standard is set on 100% of the scale and the deflection produced by the sample can be read off. Since the concentration is linearly proportional to the fluorescence, a linear scale division is sufficient. But it is different if concentration is to be determined by absorption of light. Only transmission can be detected, the light which penetrates the sample. The transmission, however, is logarithmically related to the concentration. Either the scale is divided linearly in transmission and the readings are mathematically converted or the scale is divided logarithmically in so-called absorbance values.

Absorbance is linearly related to concentration according to the Lambert—Beer-Law

$$\text{Absorbance A} = \frac{1}{\text{log transmission}}$$

$$\text{Concentration c} = \frac{\text{absorbance A}}{\text{path length d} \cdot \text{factor } \varepsilon}$$

If concentration is to be measured by determining the reflection (e.g. pigments in paint) the Kubelka—Munk-Function is applied.

$$K = \frac{\varepsilon \cdot c}{s}$$

ε = absorbance coefficient
c = concentration
s = scattering of the sample

K in turn can be computed from the measured values R with the equation

$$K = \frac{(1 - R)^2}{2_R}$$

All above mentioned laws for the concentration measurement are only correct if monochromatic light is used. The selection of the smaller or broader wavelength band which is needed for the measurement can be made by lightfilters or monochromators. It depends on the sample in question which wavelength is best suited. It will be determined in advance with pure substances or solutions in a photometer. With few exceptions the inherent constants such as absorbance coefficient ε, scattering s and wavelength at maximum absorption can be found in the appropriate literature.

Microphotometry

As the name points out, this is photometry in the microscopical range. Since in regular photometry the amounts used for measurement are in the range from some milliliters down to some microliters, microphotometry only requires amounts which are some decades smaller. Only in few cases are the samples solutions. In biology and biochemistry smears, tissue sections and suspensions are measured in transmitted light. Fluorescent samples are excited with illumination from above through the objective, measurements of colour of solid samples such as crystals and ores are also made in epi-illumination.

The instrument used in all these cases is the microscope photometer. It is different from a regular microscope in so far as the light rays coming from the specimen can be deflected on to a photodetector. The light is converted into a photocurrent and can be measured after amplification, i.e. indicated or recorded. In the case of a solution or a completely homogeneous sample the concentration in the sample can be determined by inserting a suitable light filter in the beam path of the microscope and by replacing the eyepiece by a photodetector. The measurement proper is done by using a slide on which only a drop of solvent and the coverglass is placed. By means of an amplification control the indication is set on 100% deflection. Now the solvent is replaced by the sample and it is noted how much the deflection is reduced. According to the above stated formulas and after calibration with a sample with known content, the unknown concentration can be computed.

In most cases, however, microscopical specimens are not homogeneous. A polished metal surface consists of a multitude of crystal-like grains, each of them homogeneous in itself but differing very much from its neighbours. That's why microscope photometers are equipped with diaphragm systems, so that a limited zone of the specimen can be selected and measured. Adjustable mechanical diaphragms are either round (also iris diaphragms can be considered round) or rectangular, variable from vertical slit over square to horizontal slit. Other systems are too cumbersome for common use and above all the size cannot be reproducibly adjusted. If homogeneous zones cannot be isolated, the solution is "scanning". A reproducibly adjustable diaphragm is inserted onto the microscope beam path. In commercial microscope photometers diaphragms are applied which can select areas down to 0.5 μ diameter in the object plane. In order to scan the whole specimen, the microscope is equipped with a scanning stage. This is moved by stepping motors in steps adaptable to the measured area, e.g. steps of 0.5 μ for a measured area of 0.5 μ. After every step the photodetector gets a signal which is produced by the transmission of the 0.5 μ area of the specimen. If one line is scanned the stage moves perpendicular one step and then stepwise backwards. Thus the specimen is scanned in meanders. Other scanning patterns are possible. In contrast to the measurement of larger homogeneous areas of the specimen with a resting stage, it is inconvenient to read and write down the individual values. It is more comfortable to attach a recording devide, e.g. a line recorder or better and more flexible an electronic computer. The latter combination is briefly called a "Scanning Mikroskop Photometer" (SMP).

In order to explain individual application, they may be demonstrated on the two Microscope Photometers from OPTON SMP 01 and SMP 05. In both cases the basic stand should be a stable microscope such as the Universal or the Photomicroscope (Fig. 1).

A measuring system consists of a variable diaphragm, an observation eyepiece and a photodetector place upon the microscope head. The specimen area to be measured is selected by viewing through the observation eyepiece and the diaphragm is varied until it encloses the area as well as possible. The light beam is now brought into the detector, in this case a multiplier. The photocurrent is amplified with a suitable amplifier and the value can be read from a digital indicator. This set-up is sufficient for integral measurements of homogeneous areas. But the regular stage can be replaced by a scanning stage. A separate control unit provides the commands for the stage movement. Now the measured values can be transferred either analogue to a line recorder or digital into a computer. Owners of large computers prefer to store all values in a punch tape and feed this into the computer at a later time. The direct feeding in the computer is called the "On-line-Version" and the storage in a punch-tape the "Off-line-Version". The determination of concentration requires absorbance values. The conversion of transmission into absorbance values can be done by the amplifier but in the computer as well. For reflection measurements the conversion into Kubelka—Munk values is done by the computer.

An attached line-recorder draws the density profile of one line in the specimen after another. The interpretation is difficult and therefore the hook-up of a computer is preferred. The computer is able e.g. to add up density values of area integrals which in turn can be converted into concentrations. In the SMP 01 the microscopical image is directly projected on the multiplier, only limited by the diaphragm. The instrument therefore has a high light power and is well suited e.g. for fluorescence or very dense specimens. The monochromatic illumination is obtained by a mercury vapour lamp and a suitable UV-filter in the case of fluorescence excitation, and by a tungsten lamp with an interference gradation filter in case of transmitted or reflected light measurements.

An interesting application is the colour measurement (reflection measurement) e.g. in criminal court cases. Here the stage does not move. The illuminating wavelength is varied by shifting the gradation filter in steps. The reflection of the specimen (fibers, paint chips) is continuously measured and the values are fed into a computer. The measured values are referred to a standard previously measured and the absolute colour values are computed. Usually only comparison values are required e.g. for proof of identity.

The operation principle of the SMP 05 (Fig. 2) is the same as described for the SMP 01. But there is an optical difference, since by locking in the viewing tube the total field of view can be seen. The measuring diaphragm appears as a brighter spot. It is customary, as it is in the SMP 01, to use fixed diaphragms because they permit reproducible readings. The SMO 05 can easily be equipped with an illumination system and objectives for UV-light. Particularly in biochemistry concentration measurements can be made without staining. The absorption maxime of natural products are in the UV range and they can be measured directly with the SMP 05. An image converter permits the observation of the image and the selection and adjustment of the diaphragm. It is possible to measure statically but scanning is the preferred method. The attached computer puts the incoming values in its memory at a rate of up to 50 per second. If the computer is equipped with a display screen, the shape of the specimen can be made visible after the scanning is completed. Undesired values can be extinguished. The statistical density distribution can also be displayed, it is called "histogram". According to the program the attached teletype prints the density values versus locus. The "image" of the specimen is recorded in density values. Or the teletype prints the calculated concentrations, volumes, surface areas or whatever may be desired in columns. The evaluation is determined by the program fed into the computer.

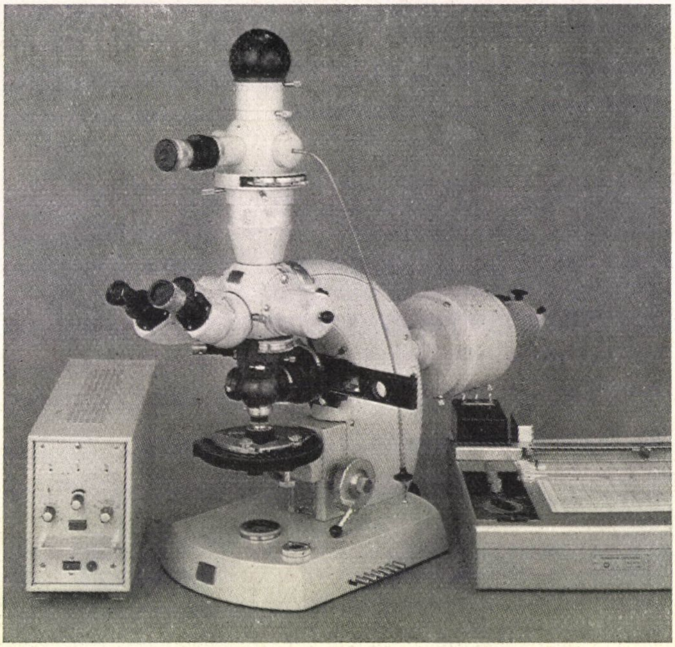

Fig. 1. OPTON Scanning Microscope Photometer Model 01. Set-up for epi-illumination

The above mentioned examples demonstrate only a few possibilities of quantitative microscopy. Not all applications are found by any means and some are at the beginning. There is a future in the evaluation of microphotographs. Pictures of chromosomes show a structure which can be measured reproducibly. Substances can be quantitatively determined by measuring autoradiographs. In aerial photographs areas with identical colours can be detected and evaluated. This must be done under a microscope since details become very small if the picture was taken from a high altitude. Other applications are the evaluation of astronomical star pictures to detect new stars and measurements on nuclear plates to detect new elementary particles. Successful measurements have been made in all these fields. With the spreading of these instruments microscope photometry will become an indispensable tool in the analytical sciences.

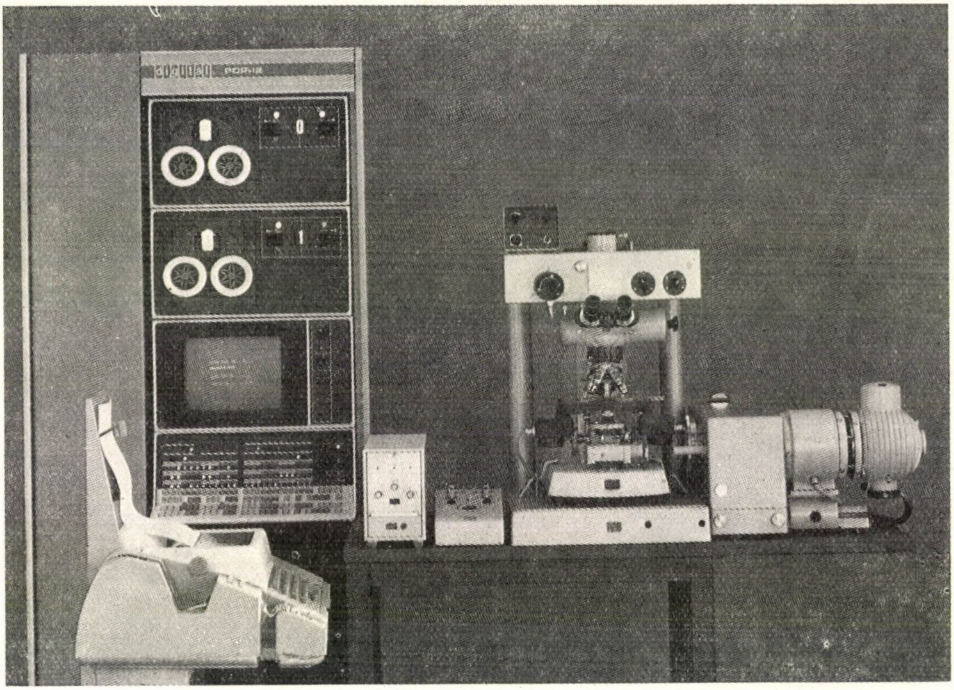

Fig. 2. OPTON Scanning Microscope Photometer Model 05 from right to left: Xenon light source, monochromator, microscope photometer, amplifier, PDP 12 computer with teletype

It was already mentioned in the introduction that certain quantitative data can be obtained from the microscopical image by means of object or eyepiece micrometers and integration plates. The whole complex of length, volume, surface and distribution measurements can be summarized under the term "stereometry". Visual methods are time consuming and need very many calculations. An instrument which can solve these problems electronically is the OPTON Micro-Videomat. In the following the basic principle shall be described.

The instrument consists of a microscope with a television camera mounted on top. The microscopical image appears on the screen of a regular black and white television receiver, connected with the camera by a cable. It is known that the television image is produced by an electron beam which runs across the screen in lines. The number of lines is known; 625 (or 525 in the case of 60 Hz main frequency). The bright-dark contrasts in the image are produced by changing the intensity of the electron beam. Every change in intensity can be used as a measuring signal.

As a simple example it may be assumed that the sample consists of fine grains of various sizes. At the edges of each grain the intensity jumps if it is intercepted by a line. When all "dark" line pieces are added up the total area of all dark particles showing up in the picture is obtained, referred to the total image area, which is automatically set on 100%. If the total magnification is known, the specimen area can be computed in mm^2 or μm^2. The instrument is also capable of counting the intercepts either if the beam enters or if it leaves the particle. These points are marked in white on the screen. Since the line width and the magnification are known, the length of the particle in vertical direction can be computed by adding up the intercepts. If the dimension of the particle in another direction is of interest the microscopical image can be rotated in the desired direction i.e. perpendicular to the television lines by means of a prism built-in the microscope head.

Fig. 3. OPTON-Micro-Videomat from right to left: Video display on top of control unit, Universal microscope with Video-tube, power supply, amplifier, Wang-computer, Teletype

Furthermore the electronics can be controlled in such a way that the lowest line which just touches the particle produces a signal. The number of signals equals the number of particles. A sophisticated circuitry prohibits a double counting at the boundary of the image if adjacent areas are to be measured.

Finally it is possible to determine particle size distribution. The images of the particles are continuously shortened, electronically of course. The images are occulted going from left to right. The smaller particles disappear first, then larger ones until only the largest ones remain. Since the control device is provided with a scale which can be calibrated, the occulting can be done in distinct steps, which correspond to preselected size classifications.

Summarized, the following basic measurements can be made: areas, lengths, numbers and size distribution. The measured values can be read off a galvanometer on the basic instrument, the seven ranges of which are switched over automatically. But all these fundamental types of measurement so far described can be made more versatile by taking the individual brightness of the specimen into account. If elements of the image on the screen have a different brightness e.g. grains of a ground metal section, darker elements can be suppressed while brighter ones are measured or vice versa. The technical term is "discrimination". Approximately ten brightness levels can be distinguished and measured.

The measurement of a single image is very seldom representative for a whole sample. It is therefore useful to attach a computer which stores and evaluates the measured data. It is obvious that not only the measured values are of interest but in addition the ratio surface to volume, mean diameters and much more. Instead of shifting the microscopical specimen to obtain more data, it is possible for the computer to control a scanning stage as described under the SMP paragraph. The functional units are the same as used with the SMP. But the specimen areas for the total measurement are of course much larger. If a stage with 10 μm stepwidth is used, the individual measurement is made after the stage has moved e.g. 10 steps. This means that the image on the screen represent 100 μm in the specimen itself. Thus a specimen area of any size can be measured without gaps or overlaps. If the specimen boundary comes into view the measurement is stopped. The stage moves 10 steps perpendicular to the scanning direction and the scanning starts backward.

In the following some applications shall be mentioned which are performed already routinely in industry:

Testing of mesh-size in manufacturing sieves and textiles,
Testing of masks for colour television tubes,
Measuring of fibers in textile industry,
Measuring of slag inclusion in rolled steel,
Measuring of percentage of perlite and ferrite in carbon steel,
Evaluation of nuclear trace photographs from nuclear reactors,
Measurements of areas and lengths on ICs.

The Micro-Videomat will be increasingly widely known and applied (like the SMP). Many measuring problems for which in the past a large number of well skilled persons had to spend much time, can now be solved fast and accurately by applying electronic devices and attached computers. And it is certain that many problems will be found and solved which seem to be far fetched at the present time.

W. Degenhardt
OPTON Feintechnik GmbH
7082 Oberkochen

GROWTH REGULATING PROPERTIES OF THE AQUEOUS EXTRACTS OF LEAVES AND FRUITS OF LANTANA CAMARA L.

In view of the vigorous vegetative and reproductive growth as well as an abundant distribution of the common shrub, *Lantana camara* L., it is believed that growth substances are largely present in different organs of this plant and consequently might serve as a potential source of hormones. Bioassay of plant growth substances has been principally confined to root tissues (Lexander 1953, Audus—Gunning 1958, Sircar—Kundu 1960, Sircar—Roy 1961) and also to seeds (MITCHELL—SKAGGS—ANDERSON 1951, BALLARD—LIPP 1959, HAMILTON—BANDURSKI—GRIGSBY 1961). Little attempt has so far been made to study the growth regulators extracted from foliar organs. Leaves are rapidly expanding tissues which may indicate their intimate connection with growth regulators. With this consideration, the present experiment was undertaken to observe whether water extracts of leaves and fruits of *Lantana camara* could exert any significant regulatory influence over the growth of rice seedlings and also to ascertain whether charcoal adsorption of the crude extract could bring any change in the mode of action of the active substances present in the extract and consequently give any indication as to the nature of the growth regulators.

Young petiolate leaves and immature fruits were collected from *Lantana camara* plants growing in the experimental garden of the Department of Botany, University of Calcutta. Two varieties of rice seeds were used, viz. dwarf variety "Taichung Native-1" and normal variety "Rupsail", both being kindly supplied by the Rice Research Station, Chinsurah, West Bengal.

Leaves and fruits after collection were immediately weighed, thoroughly washed and then blended in a "Kenmix" adding a sufficient amount of distilled water. The broth was filtered through a Buchner funnel using a thick cotton bed over the filter paper. The residue was washed several times with water and the extract was made to a definite volume. Three categories of solutions were made as follows:

(*a*) 25 g of leaves in a final volume of 400 ml,
(*b*) 100 g of leaves in a final volume of 400 ml,
(*c*) 50 g of fruits in a final volume of 400 ml.

With these solutions the following sets of experiments were performed:

(1) Effect of crude leaf extract (25 g/400 ml) and its diluted doses on seedling growth of dwarf variety of rice and the effect of the same on the normal variety.

(2) Effect of crude fruit extract and its various dilutions on seedling growth of normal variety of rice.

(3) Effect of different fractions of fruit and leaf extracts adsorbed on charcoal surface made with ether, chloroform, benzene and acetone on seedling growth of normal variety of rice.

The crude extract after the final preparation was regarded as the "basal dose". The basal dose was diluted up to 10 times with water. To each 20 ml of the basal dose (fruit 50 g/400 ml and leaf 100 g/400 ml), 2 g of activated charcoal (norite) was added, the mixture being shaken on a rotary shaker for 2 hours at room temperature and then filtered. The clear filtrate represented the "aqueous norite" fraction. Next, the adsorbed charcoal was shaken on a shaker successively with all the four solvents for 30 minutes in each case and then filtered. All these fractions were evaporated to dryness under reduced pressure and the residue in each case was dissolved in 20 ml water.

Viable rice seeds in batches after surface sterilization with 0.1 per cent mercuric chloride solution and thorough washings with either water or the desired crude extracts were then spread on Petri dishes over filter papers moistened either with water or with crude extract. In each case, water treated seeds acted as control. The seeds were allowed to germinate in a dark humid atmosphere at a constant temperature of 30°C. After 8 days of germination, the seedlings were taken out and lengths of roots and shoots recorded.

Results represent an average of five replicates. Crude water extracts of leaves and fruits of *Lantana camara* contain several substances like chlorophyll, starch, protein, fat, soluble sugars, enzymes and also growth regulators. Promotion of growth of rice seedlings by the application of such solutions may be due to the substances of nutritional value or due to the action of growth regulators. Nutritive compounds can encourage growth and usually do not retard it whereas the promotion or inhibition of plant organ growth depending upon some concentration or other are the two important functions characterizing hormones. Also the concentration of growth regulators with which shoot growth is promoted may effectively inhibit root growth. A survey of the results of the present experiment reveals all such behaviour of the extract to be specific to auxins and hence it is apparent that aqueous extracts of fruits and leaves of *Lantana camara* contain active growth regulators in addition to the other metabolites.

From Table 1 it is seen that leaf extract (25 g/400 ml) brings about a remarkable promotion of growth of the roots of dwarf rice. Promotion becomes maximum to the extent of 180 per cent over control when the basal dose is diluted 3 to 4 times. Solution diluted 10 times still brings about root promotion effectively. Promotion of shoot growth occurred as the extract was diluted more and more. In view of the observation (OGAWA 1962) that dwarf varieties of *Pharbitis nil* contain a less amount of gibberellins than normal ones, it seems likely that dwarf rice seeds also contain comparatively less gibberellins. Root promotion of dwarf rice may be explained by the assumption that the deficiency of GA was subsidized by the GA present in the leaf extract and the combined effect caused enormous increase in root length. This fantastic increase in root length treated with leaf extract can be noticed only in the dwarf variety of rice and not in the normal one. It is quite likely that some root promoting factor is operative in leaf extract which interacts with the endogenous hormones present within dwarf rice resulting in abnormal root elongation. On the other hand, the situation may be different in the normal variety and the effect becomes inhibitory causing reduction in root length (Table 2).

Curiously enough, concentrated leaf extract produced remarkable inhibition and particularly the roots were severely affected (Table 3). When treated with basal dose (100 gm/400 ml), 80 per cent of root growth was suppressed and reduction in shoot length amounted to 26 per cent. With dilution up to 10 times, shoot growth promotion was 31 per cent higher than with the control whereas root growth was still inhibited although the degree of inhibition was less as compared to that caused by the basal dose. It is seen that high concentration suppresses shoot growth whereas its dilution promotes shoot growth significantly. Also the dose which encourages shoot growth, retards root growth. This behaviour suggests the presence of indole

Table 1

Effect of leaf extract (25 g/400 ml) of Lantana camara on the growth of rice seedlings (dwarf variety)

Treatment	Increase (+) or decrease (−) as per cent control	
	Shoot	Root
Basal dose (25 g/400 ml)	−10	+141
1 : 1	− 5	+160
1 : 2	+ 3	+180
1 : 3	+ 6	+181
1 : 4	+ 7	+ 90
1 : 5	+11	+ 72
1 : 6	+16	+ 66
1 : 7	+22	+ 65
1 : 8	+24	+ 65
1 : 9	+28	+ 41

Table 2

Effect of leaf (25 g/400 ml) and fruit (50 g/400 ml) extracts on the growth of dwarf and normal varieties of rice

Treatment	Increase (+) or decrease (−) as per cent control	
	Shoot	Root
Leaf extract on dwarf	−10	+141
Leaf extract on normal	+22	− 36
Fruit extract on normal	+26	+ 15

Table 3

Effect of leaf extract (100 g/400 ml) of Lantana camara on the growth of rice seedlings (normal variety)

Treatment	Increase (+) or decrease (−) as per cent control	
	Shoot	Root
Basal dose (100 g/400 ml)	−26	−82
1 : 1	−15	−63
1 : 2	− 9	−55
1 : 3	+13	−49
1 : 4	+15	−43
1 : 5	+18	−39
1 : 6	+21	−37
1 : 7	+22	−36
1 : 8	+24	−32
1 : 9	+31	−31

auxins in the leaf extract. It is also an established fact that stems in general require high auxin concentration for growth and this promoting dose may be inhibitory to bud or root growth (THIMANN 1937, LEOPOLD 1964).

Growth response to fruit extract pinpoints more to the presence of IAA like substances which stimulate shoot and root growth, although differing in degree. Concentrated dose favours shoot growth and dilution of it encourages root growth (Table 4).

In view of the finding that rapidly growing tissues like leaves or cotyledons contain abundant GA (WHEELER 1960), it appears that the aqueous extract contains both GA and IAA along with a substantial amount of inhibitors. When the concentration of leaf extract is very high, the quantity of IAA in this solution becomes proportionately much higher than GA. In the combined action, GA effects are thus shadowed by IAA and a supraoptimal concentration of it effectively inhibits growth. According to LEOPOLD (1964), "the inhibitory action of auxins are extremely potent, especially in roots, but the promotive actions are relatively

Table 4

Effect of fruit extract (50 g/400 ml) of Lantana camara on the growth of rice seedlings (normal variety)

Treatment	Increase (+) as per cent control	
	Shoot	Root
Basal dose (50 g/400 ml)	+26	+ 8
1 : 1	+26	+15
1 : 2	+17	+19
1 : 3	+16	+18
1 : 4	+10	+26
1 : 5	+ 9	+23
1 : 6	+ 8	+22
1 : 7	+ 8	+22
1 : 8	+ 6	+21
1 : 9	+ 5	+21

Table 5

Effect of "aqueous norite" and different solvent fractions of fruit extract (50 g/400 ml) from adsorbed charcoal on the growth of rice seedlings (normal variety)

Fractions	Increase (+) or Decrease (−) as per cent control	
	Shoot	Root
Aqueous norite	+24	+60
Ether	−10	−50
Chloroform	+18	−28
Benzene	+ 4	−29
Acetone	+ 8	−36

small". Actually intermediate regions between stem and root tip are most sensitive to auxins (Furuya—Soma 1957). Also the fact that the plant extracts could serve as source of IAA was already reported earlier on the basis of the chromatographic separation of the extracts (Bennet-Clark—Kefford 1953).

Inhibitors present in fruit extract are mostly adsorbed on the surface of the activated charcoal. The aqueous phase left after their adsorption remarkably promotes root growth (Table 5). Elution of adsorbed substances by organic solvents and the applications of these fractions to rice seeds causes significant root inhibition although a little shoot promotion is noticed with these fractions (Table 5). Ether fraction caused 50 per cent root inhibition and

Table 6

Effect of "aqueous norite" fraction of leaf extract (100 g/400 ml) and its diluted doses on the growth of rice seedlings (normal variety)

Treatment	Increase (+) or decrease (−) as per cent control	
	Shoot	Root
Aqueous norite	+23	−21
1 : 1	+29	−16
1 : 3	+29	+43
1 : 5	+31	+50
1 : 7	+25	+51
1 : 9	+23	+30
1 : 99	+24	+30

appears to be the most effective solvent for the inhibitors. Adsorption of inhibitors by charcoal from nonviable rice seed extract has been shown by Dey—Sircar (1968). Ballard—Lipp (1959) extracted inhibitors from *Echium* seeds and separated them into two fractions by elutions from carbon. From the growth pattern of the present experiment, it is equally possible to consider that a substantial amount of inhibitors adsorbed on charcoal may include IAA which after elution with solvents becomes much concentrated and inhibits root growth and in the same concentration, shoot growth is promoted to some extent (Table 5). This view finds a support in the work of Bentley (1958) who concludes that hydrophilic indole conjugates are mostly adsorbed on charcoal when the water soluble component is adsorbed with it.

Promotion of shoot growth by leaf extract after charcoal adsorption reveals that most of the inhibitors are adsorbed by it. Inhibitors left still reduce root length (Table 6). Most of the inhibitors adsorbed on charcoal have got the possibility of being IAA. This view finds support in the work of Srivastava (1963) who isolated 6 auxins from the aqueous fraction of the cold ethanol extract of immature corn kernels by adsorption through stearic acid treated charcoal. It is interesting to record that the dilution of the aqueous norite fraction promotes root growth to 51 per cent, while the shoot is little affected except with the 6 times diluted dose when it shows some promotion (Table 6). This behaviour, i.e. inhibition of growth by concentrated dose and its promotion by dilution clearly establishes the presence of IAA like substances in the aqueous norite fraction.

Growth regulating activity of the eluates from the adsorbed charcoal surface reveals that ether and benzene fractions caused inhibition of both root and shoot while promotion was achieved by acetone and chloroform fractions (Table 7). Maximum root inhibition caused by the ether fraction was to the extent of 31 per cent. In the ether soluble acid fraction of nonviable rice seed extract, Dey—Sircar (1968) found the presence of IAA in supraoptimal concentration and inhibitors of the phenolic type. Also Lexander (1953) reported the presence of 3 growth regulating substances in the ether extract of wheat root. In the present experiment, growth promotion by chloroform and acetone fractions indicates the presence of GA like substances in these fractions. Although gibberellins are not soluble in non-polar solvents like chloroform, still there is evidence which indicates the solubility of known GA in chloroform and petroleum ether (Hayashi—Blumenthol-Goldsmidt—Rappaport 1962).

Table 7

Effect of "aqueous norite" and different solvent fractions of leaf extract (100 g/400 ml) from adsorbed charcoal on the growth of rice seedlings (normal variety)

Fractions	Increase (+) or decrease (−) as per cent control	
	Shoot	Root
Aqueous norite	+23	−21
Ether	− 6	−31
Chloroform	+ 2	+18
Benzene	− 8	− 5
Acetone	+12	+33

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Prepared at the Department of Botany, University of Calcutta, Calcutta.

A. K. Paul, S. Mukherji

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WATER SOLUBILITY AND PARTICLE SIZE EFFECTS OF NITRIC PHOSPHATES ON THE GROWTH AND YIELD OF WHEAT (TRITICUM AESTIVUM L.)

The sulphur shortage has led to the interest in developing processes for the manufacture of phosphatic fertilizers, which can eliminate or minimize the consumption of sulphur. Substantial progress has been made in this direction through the development of a technology utilizing nitric acid in place of sulphuric acid and manufacturing nitric phosphates. But there has been some resistance to the nitric phosphates on account of the lower water solubility of the P present. By increasing the proportion of phosphoric or sulphuric to nitric acid used products with high water solubility are possible. The economics of production of the nitric phosphates are more favourable for grades that have lower water solubility. The investigations have proved that not only fertilizers containing water soluble phosphates show a good promise, other forms of phosphates which are soluble in citrate or citric acid may be equally effective in supplying phosphorus to the plants. **Rogers** (1951) showed that phosphorus in nitric phosphates differing in water solubility was as available as concentrated superphosphate and commercial ammoniated mixtures to corn, cotton and small grains. There was no evidence that high water solubility (greater than 10%) was required in the nitric phosphates for small grains, corn and cotton. **Sharma—Singh** (1967) reported similar findings on wheat in Karnal (India) soils. **Gautam** *et al.* (1967) reported similar responses in irrigated wheat to superphosphate, ODDA and PEC nitric phosphates at 6 of 18 centres where P_2O_5 response was obtained. **Atanasiu** (1971) reported similar results with water and citrate soluble phosphatic fertilizers on two alluvial soils from West Turkey (pH more than 7) and two from Ethiopia (pH 5.1 and 4.1). The effects on the yield of the two forms were adequate on all the soils. On Ethiopian soils the phosphorus uptake from the citrate soluble phosphate was higher than that from water soluble phosphate. The Fertilizer Corporation of India Ltd., Sindri, conducted a large number of field trials with nitric phosphates on wheat, potato and maize covering the major soil types and repeated over a number of years (**Dhua** 1971a). The results revealed that responses with nitrophosphate were generally similar or superior as compared with other straight fertilizers. In another study with nitric phosphates by the Fertilizer Corporation of India, Ltd., Sindri, using wet land paddy as the test crop, results quite clearly indicated that under water logged paddy field conditions there was no difference between citrate and water soluble phosphates (**Dhua** 1971b). Related closely to water solubility is the granule size of the fertilizers which also influences greatly the phosphorus availability to plants. To obtain information about the water solubility and granule size effects of nitric phosphates on a calcareous soil and the availability of these fertilizers to the wheat crop a greenhouse experiment was conducted at Pantnagar, U.P. (India).

A soil testing ‘low’ in available P (3.2 kg of available P/ha-Olsen’s method) collected from plot 61, Block D, Pantnagar Farm was used for the study. The soil is not yet classified at the series level. The soil is a Mollisol. The soils in the area are derived from calcareous alluvium

Table 1

Sources of phosphorus used in the study

S. No.	Fertilizer	Grade N P K per cent	Water solubility (%)	Granule size (US Tyler mesh)	Major compounds present
1.	Nitric phosphate	20 8.80 0	23.0	— 6 + 9	$CaHPO_4$, $NH_4H_2PO_4$, $Ca(NH_3)_2$ reprecipitated apatite
2.	Nitric phosphate	-do-	23.0	— 9 + 12	
3.	Nitric phosphate	-do-	23.0	—12 + 20	
4.	Nitric phosphate	-do-	40.0	— 6 + 9	
5.	Nitric phosphate	-do-	40.0	— 9 + 12	
6.	Nitric phosphate	-do-	40.0	—12 + 20	
7.	Nitric phosphate	-do-	56.6	— 6 + 9	
8.	Nitric phosphate	-do-	56.6	— 9 + 12	
9.	Nitric phosphate	-do-	56.6	—12 + 20	

from the Himalaya mountains. The bulk soil was screened through a 2 mm sieve and 4 kg of it was potted in a polyethylene bucket (5 kg capacity). The sources (Table 1) were applied at two rates —25 and 50 ppm P, replicating each treatment three times. There was one control treatment (no P). Nitrogen at the rate of 120 kg N/ha and potassium at the rate of 25 kg K/ha were applied uniformly to all the pots through reagent grade NH_4Cl and KCl, respectively. Fifteen seeds of wheat (variety 'Sonalika') were sown in each pot and later thinned to ten plants per plot. Water was maintained at field capacity to 75 per cent field capacity throughout the growth period. At the end of 45 days, the above ground parts were cut, dried at 65°C and the dry weight was recorded. One gram of finely ground dried plant material was added to a 50 ml beaker and ignited at 550°C for four hours in a muffle furnace. The ash was dissolved in 100 ml of 0.3 N HNO_3. Aliquots of this solution were employed for the determination of phosphorus by the molybdoranadate method (Jackson 1958). A representative soil sample was taken from each pot and analysed for available phosphorus by Olsen's method (Olsen *et al.* 1954).

All the fertilizer P treatments resulted in higher dry matter and phosphorus accumulation by the wheat plants, and soil available phosphorus (determined after the harvest of the crop) in comparison to the control (Table 2). The 50 ppm P application rate was significantly superior to the 25 ppm P application rate for all the parameters studied (Table 3). The highest dry matter production at both the levels of application was obtained with the nitric phosphate of 23 per cent water solubility and —9 + 12 granule size. The lowest dry matter production at 25 ppm application was obtained with nitric phosphate of 56.6 per cent water solubility and —12 + 20 granule size, whereas at 50 ppm application the same was obtained with nitric phosphate of 56.6 per cent water solubility and —9 + 12 granule size, and nitric phosphate of 40 per cent water solubility and —6 + 9 granule size. The highest phosphorus accumulation at both the levels was obtained with nitric phosphate of 23 per cent water solubility and —12 + 20 granule size. The lowest phosphorus accumulation at 25 ppm application was obtained with nitric phosphate of 56.6 per cent water solubility and —12 + 20 granule size, whereas at 50 ppm application the same was obtained with nitric phosphate of 40 per cent water solubility and —9 + 12 granule size.

The highest soil available phosphorus at both the levels of application was obtained with nitric phosphate of 56.6 per cent water solubility and —6 + 9 granule size. The lowest content at 25 ppm application was obtained with nitric phosphate of 40 per cent water solubility and —6 + 9 granule size, whereas at 50 ppm application the same was obtained with nitric phosphate of 40 per cent water solubility and —9 + 12 granule size (Table 2).

Table 2

Dry matter yield, phosphorus uptake by wheat and available phosphorus content in soil treated with various phosphorus sources

S. No.	Fertilizer	Dry matter yield (gms)		Phosphorus uptake (mg per pot)		Available phosphorus content of soil in (kg P/ha)	
		25 ppm P	50 ppm P	25 ppm P	50 ppm P	25 ppm P	50 ppm P
1.	Nitric phosphate — 6 + 9 mesh (23% W.S.)	16.50	21.3	46.00	64.67	18.33	35.50
2.	-do- — 9 + 12 ,,	19.20	21.8	50.13	56.61	23.16	32.00
3.	-do- —12 + 20 ,,	19.00	21.3	50.66	66.77	14.33	27.66
4.	Nitric phosphate — 6 + 9 ,,	16.80	19.2	42.42	52.94	7.16	19.66
5.	-do- — 9 + 12 ,,	17.20	20.0	44.50	50.71	10.00	16.00
6.	-do- —12 + 20 ,,	15.20	20.2	34.62	54.34	24.83	24.16
7.	Nitric phosphate — 6 + 9 ,,	13.80	21.3	35.64	57.13	33.66	46.16
8.	-do- — 9 + 12 ,,	14.30	19.2	34.18	52.16	27.50	34.50
9.	-do- —12 + 20 ,,	12.60	20.6	32.79	53.42	20.16	37.66
10.	Control (No phosphorus)	4.50		8.69		4.66	
	'F' test	23.56*		25.95*		3.92*	
	S.Em.±	0.20		0.60		1.27	
	C.D.	0.76		2.28		4.83	

* = Significant at the 5 per cent level of probability. F values are calculated values

The production of dry matter varied with the water solubilities of the nitric phosphates and increased with decreasing water solubility. For phosphorus uptake, the 23 per cent water solubility was found to be significantly superior to the 40 and 56.6 per cent water solubilities, whereas the 40 and 56.6 per cent water solubilities did not differ significantly from each other. The total phosphorus uptake again increased with decreasing water solubility (Table 3). Rogers (1951) compared two nitric phosphates of 10 and 40 per cent water solubilities. The evidence was strong that the nitric phosphate of higher water solubility was inferior to the nitric phosphate of lower water solubility, based on the results from 16 tests in seven different states.

In the case low water soluble phosphorus fertilizers, the reaction of the phosphorus with the soil to form unavailable products is likely to be slow compared to the high water soluble fertilizers as the rate of phosphorus dissolution in the soil is low in the former case. Therefore, low water soluble fertilizer would result in a steady supply of phosphorus to the growing plants throughout the crop growing season. On the other hand the high water soluble sources will result in a good supply of phosphorus in the early stages and will fail to supply phosphorus adequately to plants at the later period of plant growth. Such a behaviour is quite obvious from the interaction observed between the rate and water solubility and the dry matter production and phosphorus accumulation of the plants (Table 4a). The highest dry matter yield and phosphorus uptake were observed with the 23 per cent water solubility at 50 ppm P application rate, whereas the lowest values were observed with the 56.6 per cent water solubility

Table 3

Effect of water solubility, granule size and application rate of nitric phosphates on dry matter and phosphorus accumulation by wheat and available phosphorus in soil

Treatment	Dry matter (g)	Phosphorus uptake (mg/pot)	Available phosphorus in soil (kg/ha)
Water solubility			
23%	19.86	55.81	25.16
40%	18.08	46.59	16.97
56.6%	17.00	44.22	33.27
S.Em.±	0.36	1.10	2.32
C.D. at 5%	1.03	3.14	6.66
Granule size			
— 6 + 9	18.16	49.80	26.75
— 9 + 12	18.61	48.05	23.86
—12 + 20	18.16	48.77	24.80
S.Em.±	0.36	1.10	2.32
C.D. at 5%	—	—	—
Rate			
25 ppm P	16.	41.22	19.90
50 ppm P	20.55	56.53	30.37
S.Em.±	0.29	0.90	1.90
C.D. at 5%	0.83	2.57	5.45

at the 25 ppm P application rate. At the 56.6 per cent water solubility, the 25 ppm P level being more water soluble as well as a smaller quantity was not sufficient to meet the phosphorus requirements of the plants, but at the 23 per cent water solubility a 50 ppm phosphorus application, besides a slow release, ensured a greater supply of phosphorus.

Also there was significant interaction between the application rate and granule size and the phosphorus uptake of the plants (Table 4b). The highest phosphorus uptake was obtained with —6 + 9 granule size at 50 ppm P application rate, whereas the lowest uptake was obtained with —12 + 20 granule size at the 25 ppm application rate. The large granules, having a small surface area per unit of applied P, ensure less contact with the soil: thus, their phosphorus content is less prone to reactions with the soil. Therefore, at the 50 ppm application rate, besides less formation of unavailable reaction products from large granules, there was an advantage of a larger quantity of phosphorus. Small granules react extensively with the soil lose their phosphorus content especially very readily, especially so at the lower level of application. The granule size of nitric phosphates per se did not have any significant effect on any of the parameters studied (Table 4b).

Table 4a

Interaction between application rate and water solubility of nitric phosphates as related to dry matter production and phosphorus uptake by wheat plants

Rate	Dry matter yield of wheat (g) as affected by rate and water solubility	
	25 ppm P	50 ppm P
Water solubility		
23%	18.22	21.50
40%	16.39	19.77
56.6%	13.61	20.39

S.Em. ± = 0, C.D. at 5% = 1.46.

Rate	Phosphorus uptake by wheat (mg/pot) as affected by rate and water solubility	
	25 ppm P	50 ppm P
Water solubility		
23%	48.93	62.75
40%	40.51	52.66
56.6%	34.01	54.24

S.Em. ± = 1.55, C.D. at 5% = 4.42.

Table 4b

Interaction between application rate and granule size of nitric phosphates as related to phosphorus uptake by wheat

Rate	Phosphorus uptake by wheat (mg/pot) as affected by rate and granule size	
	25 ppm P	50 ppm P
Granule size		
− 6 + 9	41.35	58.24
− 9 + 12	42.94	53.16
−12 + 20	39.36	58.18

S.Em. ± = 1.55, C.D. at 5% = 4.42.

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P. D. Sharma, T. A. Singh

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CAUSTIC HYDROLYSIS AND PHYSICO-CHEMICAL PROPERTIES OF BEEF FATS

Fats are one of the major components in the carcass of a meat animal, and are exceeded only by the water and protein contents. Edible animal fats in U.A.R. are obtained essentially from beef, buffalo and sheep, and to a lesser extent from camel and goat carcasses. Lard is utilized only in a small amount.

Fats can be produced by using various processes. In most cases the fatty tissues are cooked, and fat is released by the high temperature and cell rupture. Wet rendering (Dean 1938, Hefter—Schonfeld 1939, Hilditch 1941 and Jacobs 1951) may be carried out in a simple open tank equipped with a low speed agitator and a steam-heated jacket. The fatty material is placed in the tank and heated with water addition, until the major part of the fat forms a clear layer on the surface, where it can be drawn off.

The rendering in open kettle is relatively slow and inefficient and, in addition, subjects the fat to a certain amount of undesirable oxidation as a result of its contact with the air at high temperature. The caustic hydrolysis method of fat rendering has been recommended by several workers, e.g. Liberman—Mirkin (1952), Liberman—Jontar (1957), and Liberman—Petroviski (1960).

The physical and chemical properties of fats of local beefs were dealt with by Habib—Youssef (1966) and Moursy *et al.* (1966). Rendered beef fat differs in quality according to the part of the animal from which it is obtained and the rendering process itself. This investigation was carried out to clarify the effects of caustic hydrolysis rendering on the yield and quality of rendered beef fats.

Table 1

*Chemical composition of raw beef tallows**

Source of fat	Moisture, %	Protein, %	Crude fat, %	Ash, %
Perinephric fat	20.90	4.24	73.86	1.00
Mesenteric fat	29.40	4.96	64.78	0.89

* Each figure given in this table is a mean of three determinations.

Table 2

Average results of the influence of 30% alkali and alkali mixtures on the percentages of perinephric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	7.5	69.42
2	0.8 NaOH (A)	0.017	0.24	1.5	70.80
3	1.2 NaOH	0.025	0.36	1.5	71.86
4	1.6 NaOH	0.034	0.48	1.5	71.92
5	2.0 NaOH	0.042	0.60	1.5	*72.75*
6	2.4 NaOH	0.050	0.72	1.5	71.82
7	1.0 Na_2CO_3 (B)	0.021	0.30	5.0	70.50
7	1.5 Na_2CO_3	0.032	0.45	5.0	71.42
9	2.0 Na_2CO_3	0.042	0.60	5.0	71.80
10	2.4 Na_2CO_3	0.050	0.72	4.5	*72.20*
11	2.6 Na_2CO_3	0.055	0.78	4.0	71.10
12	0.8 A + 1.0 B	0.017 + 0.021	0.24 + 0.30	5.0	70.45
13	1.2 A + 1.5 B	0.025 + 0.032	0.36 + 0.45	5.0	*72.10*
14	1.5 A + 2.0 B	0.034 + 0.042	0.48 + 0.60	5.0	72.00
15	2.0 A + 2.4 B	0.042 + 0.050	0.60 + 0.72	4.0	71.80
16	2.4 A + 2.6 B	0.050 + 0.055	0.72 + 0.78	4.0	71.00

The tallow samples used in this experiment were collected from mesenteric and perinephric fat of cows (approximately 5 years old). Samples were taken from freshly dressed carcasses and transferred to the laboratory with minimum time delay. The visible fat after being thoroughly minced, was rendered by the wet rendering method. Rendering was carried out in pyrex test tubes using a low benzene flame. Three alkali solutions in several concentrations, namely; 0.8, 1.2, 1.6, 2 and 2.4% NaOH (*A*) :1, 1.5, 2, 2.4 and 2.6% Na_2CO_3 (*B*): and 0.8 + 1, 1.2 + 1.5, 1.6 + 2, 2 + 2.4 and 2.4 + 2.6 alkali mixture (*A* + *B*) were used in this

Table 3

Average results of the influence of 40% alkali and alkali mixtures on the percentages of perinephric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	7.5	69.42
2	0.8 NaOH (A)	0.022	0.32	1.5	70.14
3	1.2 NaOH	0.034	0.48	1.5	70.90
4	1.6 NaOH	0.045	0.64	1.5	71.80
5	2.0 NaOH	0.056	0.80	1.5	*72.20*
6	2.4 NaOH	0.067	0.96	1.5	71.08
7	1.0 Na_2CO_3 (B)	0.028	0.40	5.0	70.40
8	1.5 Na_2CO_3	0.042	0.60	5.0	71.20
9	2.0 Na_2CO_3	0.056	0.80	5.0	71.60
10	2.4 Na_2CO_3	0.067	0.96	4.0	*72.00*
11	2.6 Na_2CO_3	0.073	1.04	4.0	71.00
12	0.8 A + 1.0 B	0.022 + 0.028	0.32 + 0.40	5.0	70.14
13	1.2 A + 1.5 B	0.034 + 0.042	0.48 + 0.60	5.0	71.18
14	1.6 A + 2.0 B	0.045 + 0.056	0.64 + 0.80	5.0	*71.90*
15	2.0 A + 2.4 B	0.056 + 0.067	0.80 + 0.96	4.0	71.20
16	2.4 A + 2.6 B	0.067 + 0.073	0.96 + 1.04	4.0	69.90

study. These three alkali solutions were utilized in different quantities, i.e. 30, 40 and 50% from the crude fat weight, for fat extraction.

The hydrolysis of minced raw fat was conducted as above mentioned. The molten was collected at intervals, then filtered hot. The fat yield was recorded in each experiment while samples from the filtered fats were used in estimating the major components and the chemical and physical constants of the two afore-mentioned fats.

The physical and chemical constants of fats were carried out as follows. The specific gravity was determined by means of pycnometer and was reported as sp. gr. $^{60}_{25}$ according to the procedure given by Trubold—Aurand (1963). The melting point was determined by the capillary tube method recommended by Trubold—Aurand (1963). A thin-walled capillary tube, approximately 8 cm long and 1 mm in diameter was used. The solidification point was determined according to the method described by Sokolov (1953). While the iodine number, the acid number, and the peroxide number were determined by standard methods described by the A.O.A.C. (1960).

The quantitative analysis of the proximate components of perinephric and mesenteric fats, i.e. moisture, protein, fat and ash, was performed according to the corresponding methods described by A.O.A.C. (1960).

Chemical composition of beef fat. The results presented in Table 1 indicate the mean

Table 4

Average results of the influence of 50% alkali and alkali mixtures on the percentages of perinephric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	7.5	69.42
2	0.8 NaOH (A)	0.028	0.40	1.5	70.00
3	1.2 NaOH	0.042	0.60	1.5	70.30
4	1.6 NaOH	0.056	0.80	1.5	*72.10*
5	2.0 NaOH	0.070	1.00	1.5	71.80
6	2.4 NaOH	0.084	1.20	1.5	71.00
7	1.0 Na_2CO_3 (B)	0.035	0.50	5.0	70.30
8	1.5 Na_2CO_3	0.053	0.75	5.0	71.10
9	2.0 Na_2CO_3	0.070	1.00	4.0	*71.90*
10	2.4 Na_2CO_3	0.084	1.20	4.0	70.80
11	2.6 Na_2CO_3	0.091	1.30	4.0	70.00
12	0.8 A + 1.0 B	0.028 + 0.035	0.40 + 0.50	5.0	70.10
13	1.2 A + 1.5 B	0.042 + 0.053	0.60 + 0.75	4.0	*71.80*
14	1.6 A + 2.0 B	0.056 + 0.070	0.80 + 1.00	4.0	71.60
15	2.0 A + 2.4 B	0.070 + 0.084	1.00 + 1.20	4.0	70.00
16	2.4 A + 2.6 B	0.084 + 0.091	1.20 + 1.30	4.0	68.80

values of the major chemical components of beaf tallows taken from perinephric and mesenteric fat depots. On the whole, there were marked differences between the perinephric and mesenteric beef fats. The results revealed that the mesenteric fatty tissues proved to contain less crude fat and ash than the perinephric ones. Meanwhile, the moisture level was higher in the former than in the latter. The differences in protein content of these fat depots were not marked.

Crude fat yield. The average results of the influence of 30%, 40% and 50% sodium hydroxide solutions, sodium carbonate solutions and alkali mixture solutions on the basis of the weight of raw fat are given in Tables 2, 3 and 4, 5, 6 and 7 for perinephric and mesenteric fat, respectively. The highest recorded perinephric fat yields for 30, 40 and 50% alkali quantity are presented in the Tables 2, 3 and 4. Meanwhile, the highest recorded mesenteric fat yields for 30, 40 and 50% alkali quantities are presented in Tables 5, 6 and 7.

These results were further illustrated by statistical analysis (Snedecor 1956); given in Table 8. The statistical analysis revealed that the calculated t value was 14.50 in the case of perinephric fat, and thus much higher than $t_{0.5}$ and $t_{0.1}$ values (4.303 and 9.925, respectively). This means that the perinephric fat recovery by the caustic hydrolysis method of rendering is highly significant. While, the calculated t value was 5.97 in the mesenteric fat, which is rather high than $t_{0.5}$ value (4.303) and much lower than $t_{0.1}$ (9.925). This indicates that the mesenteric fat recovery by the caustic hydrolysis method is only significant at the level $t_{0.5}$.

Table 5

Average results of the influence of 30% alkali and alkali mixtures on the percentages of mesenteric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	8.0	60.89
2	0.8 NaOH (A)	0.017	0.24	1.5	61.88
3	1.2 NaOH	0.025	0.36	1.5	62.80
4	1.6 NaOH	0.034	0.48	1.5	62.90
5	2.0 NaOH	0.042	0.60	1.5	*63.80*
6	2.4 NaOH	0.050	0.72	1.5	61.90
7	1.0 Na_2CO_3 (B)	0.021	0.30	5.0	61.10
8	1.5 Na_2CO_3	0.032	0.45	5.0	61.90
9	2.0 Na_2CO_3	0.042	0.60	5.0	62.00
10	2.4 Na_2CO_3	0.050	0.72	4.5	*63.00*
11	2.6 Na_2CO_3	0.055	0.78	4.5	61.20
12	0.8 A + 1.0 B	0.017 + 0.021	0.24 + 0.30	5.0	61.00
13	1.2 A + 1.5 B	0.025 + 0.032	0.36 + 0.45	5.0	*62.80*
14	1.6 A + 2.0 B	0.034 + 0.042	0.48 + 0.60	5.0	62.00
15	2.0 A + 2.4 B	0.042 + 0.050	0.60 + 0.72	4.5	62.12
16	2.4 A + 2.6 B	0.050 + 0.055	0.72 + 0.78	4.5	61.65

Physical and chemical characteristics of rendered beef fat. The results obtained were summarized, and the mean analytical values of the physical and chemical characteristics of the depot fat of beef are presented in Table 9. The alkali concentrations giving the highest yields, namely 2.0% NaOH (30% on the basis of the weight of raw fat), 2.0% NaOH (40%), and 1.6% NaOH (50%) were chosen for physical and chemical determinations.

The specific gravity of perinephric fat ranged from 0.8800 to 0.8809, while that of mesenteric ranged from 0.8572 to 0.8577. The specific gravity of fats obtained by the different alkali treatments did not vary. The melting point and the solidification point showed similar trends. However, the perinephric fat gave lower values than the mesenteric fat (Table 9). Usually, the solidification point was lower than the melting point of each fat. On the other hand, the mean recorded iodine values of the two fats showed little variation. The lower value is that of the mesenteric (38.78), while the higher is that of perinephric fat (40.20). On the whole, only slight variations could be detected between the fats obtained as a result of different alkali treatments.

Besides, the results shown in Table 9 indicate that there were no marked differences between the acid value and peroxide value of the two fats.

The various fat values have been determined for obtaining information on the industrial possibilities of beef tallows. These tallows are commonly used by the lower income group of

Table 6

Average results of the influence of 40% alkali and alkali mixtures on the percentages of mesenteric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	8.0	60.89
2	0.8 NaOH (A)	0.022	0.32	1.5	61.70
3	1.2 NaOH	0.034	0.48	1.5	62.60
4	1.6 NaOH	0.045	0.64	1.5	62.70
5	2.0 NaOH	0.056	0.80	1.5	*63.20*
6	2.4 NaOH	0.067	0.96	1.5	61.68
7	1.0 Na_2CO_3 (B)	0.028	0.40	5.0	61.05
8	1.5 Na_2CO_3	0.042	0.60	5.0	61.70
9	2.0 Na_2CO_3	0.056	0.80	5.0	61.90
10	2.4 Na_2CO_3	0.067	0.96	4.0	*62.80*
11	2.6 Na_2CO_3	0.073	1.04	4.0	61.15
12	0.8 A + 1.0 B	0.022 + 0.028	0.32 + 0.40	5.0	60.95
13	1.2 A + 1.5 B	0.034 + 0.042	0.48 + 0.60	5.0	*62.10*
14	1.6 A + 2.0 B	0.045 + 0.056	0.64 + 0.80	5.0	61.90
15	2.0 A + 2.4 B	0.056 + 0.067	0.80 + 0.96	4.0	61.95
16	2.4 A + 2.6 B	0.067 + 0.073	0.96 + 1.04	4.0	61.20

population in U.A.R., as a substitute for ghee. The results showed that the mesenteric fatty tissues proved to contain less crude fat and ash, and more moisture than the perinephric fat, which corresponds favourably to the data reported by Liberman—Petroviski (1960). In addition, the following alkali solutions, namely 30% (2.0 NaOH), 40% (2.0 NaOH), and 50% (1.6 NaOH) rendered the highest fat yields: 72.75, 72.20 and 72.10% for perinephric fat, and 63.80, 63.20 and 62.50 for mesenteric fat, respectively (Table 8).

The statistical analysis revealed that the recovery of perinephric fat by the caustic hydrolysis method of rendering is more significant than that of the mesenteric fat. The results recorded for the mean values of physical and chemical characteristic of beef fats were found to be in good accordance with those obtained by Liberman—Petroviski (1960) and Habib—Youssef (1966). Upon comparison of these values, it is possible to observe similarity in some characteristics.

Results of specific gravity tests show that the perinephric fat contains a somewhat larger proportion of unsaturated fatty acids in the glycerides than the mesenteric fat. Since the unsaturated fatty acids commonly occurring as the components of edible fats are liquid at room temperature, the iodine value can be related to the melting point or the hardness of the beef fats. This is found to be true for both perinephric and mesenteric fats, and agrees with the findings given by Moursy *et al.* (1966) for beef depot fats. The higher melting point of mesenter-

Table 7

Average results of the influence of 50% alkali and alkali mixtures on the percentages of mesenteric fat yield of beef

Exp. No.	Alkali concentration, %	Alkali quantity: raw fat weight		Time of hydrolysis	Fat yield
		g	%	(hrs)	%
1	0.0 alkali	0.0	0.0	8.0	60.89
2	0.8 NaOH (A)	0.028	0.40	1.5	61.60
3	1.2 NaOH	0.042	0.60	1.5	62.20
4	1.6 NaOH	0.056	0.80	1.5	*62.50*
5	2.0 NaOH	0.070	1.00	1.5	62.30
6	2.4 NaOH	0.084	1.20	1.5	61.95
7	1.0 Na_2CO_3 (B)	0.035	0.50	5.0	61.00
8	1.5 Na_2CO_3	0.053	0.75	5.0	61.40
9	2.0 Na_2CO_3	0.070	1.00	5.0	61.50
10	2.4 Na_2CO_3	0.084	1.20	4.0	*62.30*
11	2.6 Na_2CO_3	0.091	1.30	4.0	61.10
12	0.8 A + 1.0 B	0.028 + 0.035	0.40 + 0.50	5.0	60.80
13	1.2 A + 1.5 B	0.042 + 0.052	0.60 + 0.75	5.0	*62.00*
14	1.6 A + 2.0 B	0.056 + 0.070	0.80 + 1.00	5.0	61.80
15	2.0 A + 2.4 B	0.070 + 0.084	1.00 + 1.20	4.0	61.60
16	2.4 A + 2.6 B	0.084 + 0.091	1.20 + 1.30	4.0	61.10

ic fat compared to that of perinephric gives rise to preferring mesenteric beef for frying purposes, especially for frying of meat cuts taken from old cows, since the latter needs further cooking.

Likewise, the higher melting point and the saturated and unsaturated fatty acid contents of mesenteric would make it suitable to be used in the injection of the tough meat for tenderization purposes, as it was previously reported by Youssef (1965). In addition, there were no marked variations between the above-mentioned fats in the acid value and the peroxide values. An overall look at Table 9 indicates that the physical and chemical characteristics of mesenteric fat prove its further suitability to be mixed with hydrogenated vegetable oils in shortening production. Such products would improve the quality of baked products and prolong their life.

The results revealed that the alkali hydrolysis method had no actual effect on the physical and chemical properties of beef fats. Such a treatment, however, resulted in a marked increase in the fat yields. For instance, the following alkali solutions, namely 30% (2.0 NaOH), 40% (2.0 NaOH), and 50% (1.6 NaOH) rendered 98.5%, 97.7% and 97.6% perinephric fat: and 98.4 % 97.5% and 96.4% mesenteric fat of its total crude content, instead of 94.4% and 94.0% rendered by the conventional method of fat rendering in open kettles (Tables 1 and 8).

On the other hand, in the light of the results already obtained, the advantages of caustic hydrolysis rendering process may be summed up as follows; *a)* Higher yields of first

Table 8

Average percentages of fat recovery in perinephric and mesenteric fat of beef (Statistical analysis of t test results)

Treatment	Hydrolysis method values (X_1)	Normal rendering method (X_2) (Control)	$X = (X_1 - X_2)$	D^2
A) *Perinephric:*				
1. 30% (2.0 NaOH)	72.75	69.42	3.33	11.089
2. 40% (2.0 NaOH)	72.20	69.42	2.78	7.728
3. 50% (1.6 NaOH)	72.10	69.42	2.68	7.182
Total:	217.05	208.26	8.79	25.999
$\bar{x}$	72.35	69.42	2.93	
S $\bar{d}$	0.202			
t	14.50			
t 0.5	4.303			
t 0.1	9.925			
B) *Mesenteric:*				
1. 30% (2.0 NaOH)	63.80	60.89	2.91	8.468
2. 40% (2.0 NaOH)	63.20	60.89	2.31	5.336
3. 50% (1.6 NaOH)	62.50	60.89	1.61	1.592
Total:	189.50	182.67	6.83	15.396
$\bar{x}$	63.16	60.89	2.27	
S $\bar{d}$	0.38			
t	5.97			
t 0.5	4.303			
t 0.1	9.925			

grade edible fats, *b)* attractive fuel (steam) savings, and *c)* considerable reduction in time of rendering.

Furthermore, the free fatty acids in the fresh and caustic rendered beef fat were rather similar. The values indicated very little hydrolysis in the fatty tissues. The peroxide values were low, showing that only a little oxidation of the fat had taken place.

*

Prepared at the Department of Food Technology and Department of Food Control Assiut University, Assiut.

K. E. YOUSSEF, A. Y. LOTFI, A. A. ESMAIL

Table 9

Average results of physical and chemical constants for perinephric and mesenteric fat of beef

Fat constants	Control		30% (2.0 NaOH)		40% (2.0 NaOH)		50% (1.6 NaOH)	
	P	M	P	M	P	M	P	M
Physical								
1. Specific gravity	0.8809	0.8578	0.8800	0.8577	0.8801	0.8572	0.8803	0.8575
2. Melting point	47.00	49.25	47.10	49.46	47.05	49.48	47.00	49.46
3. Solidification point	38.60	39.10	38.50	39.20	38.55	39.60	38.65	39.40
Chemical								
1. Iodine value	40.15	38.92	40.10	38.82	40.12	38.78	40.20	38.80
2. Acid value	0.21	0.22	0.21	0.21	0.20	0.22	0.21	0.20
3. Peroxide value	0.60	0.64	0.62	0.66	0.64	0.66	0.66	0.66

N. B.:
P = perinephric fat
M = mesenteric fat

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SOME QUESTIONS OF LEAF ORGANIZATION FROM THE POINT OF VIEW OF INITIATION, FORM AND STRUCTURE

Details on the different stages of leaf organization are found rather scattered in the literature. This made it justified to give a brief summarization of the question considered important from the point of view of research as well. Within the question of leaf organization the histogeny of leaves of *Pteridophyta*, *Gymnospermae* was discussed by GUTTENBERG (1961, 1966), while the development of leaves in *Angiospermae* was treated comprehensively by GUTTENBERG (1960), KAUSSMAN (1963) and ESAU (1969). On leaf formation detailed data were presented by DITTMER (1964). In the present paper leaves of a species of each of *Pteridophyta*, *Gymnospermae* and *Angiospermae* are described from morphological and histological points of view, placed in a sequence of organization advancing toward the more developed form, often irrespective of the taxonomic classification.

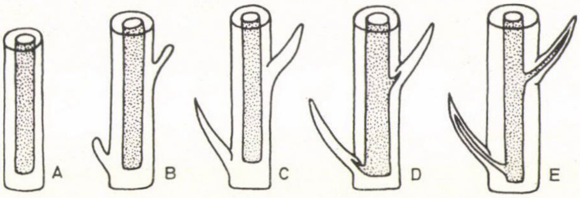

Fig. 1. Enation theory of the origin of leaf

The examinations were performed partly on the basis of data available in the literature, partly leaves of the collected plant species were fixed in Bouin's fixing solution, embedded in paraffine with the usual microtechnical method, then the prepared section series was stained with Ehrlich's haematoxiline. Of the characteristic tissue structures drawings were made.

The first stemmed (shooted) plants were leafless, and assimilation was performed by the green shoot axis itself. As in the case of algae, in the course of development with the growing surface the plants took a leaf-like structure in order to be able to perform the new function of assimilation, this increase of surface occurred early in the terrestrial plants, not only in the form of the telomes developing new branches but also by the formation of leaves.

As for the appearance of leaves more than one view has been formed. According to one of them the leaves developed on the surface of the shoot as emergences. These leaves were spike-like and there was not connection of vascular bundles between the leaves and the shoot. In leaves of higher organization though still developing as a surface formation a vascular bundle running to the leaf apex is formed. This is the so-called enation theory (Fig. 1). According to another opinion the leaves developed from the erect, dichotomically branching leafless shoots parallel with the sympodial branching. In this case one of the two prongs of the bifurcated branch continued developing in the usual way, while the other fell back in development, flattened and was transformed into a leaf, e.g. in the *Lycopodium* species (Fig. 2). These two types of leaf are of small size, so-called microphyllum. In the third view the leaves developed in the following way; on the protophyte of dichotomic branching, the telomes and the underlying mesomes shortened, became level, leaf-like and grew together at the edges. This way, by so-called planation, petioled leaves with dichotomic vein pattern developed. This type of leaf organization can be observed in fossile and living ferns (Fig. 2/B). This type of leaf is called macrophyllum, due to its size.

According to the fourth view of leaf development the leaves appeared earlier than the shoot apex and the root-system (leaf theory, Fig. 3). The development of this leaf type can be traced back to the highly lamelliform algae. This theory has the least probability. The enation theory and the two telome theories are the most acceptable. The evolution of leaves probably took place in a number of different ways.

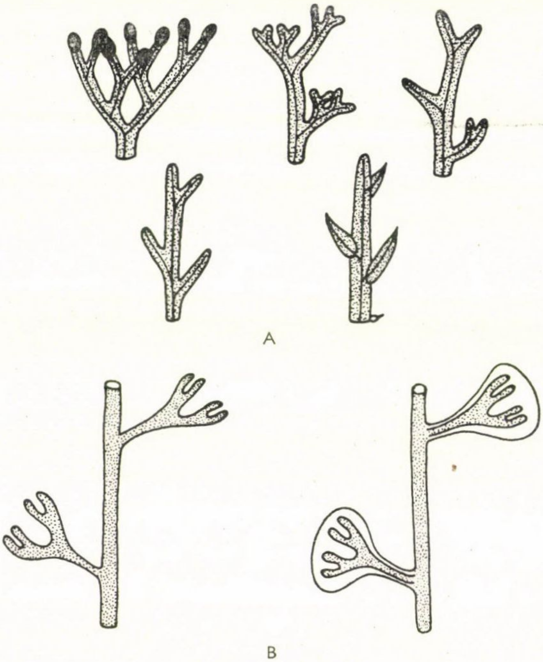

Fig. 2. Origin of leaf through the transformation of bifurcate shoot axes. *A* in *Lycopodiaceae*, *B* in *Polypodiaceae* (planation theory)

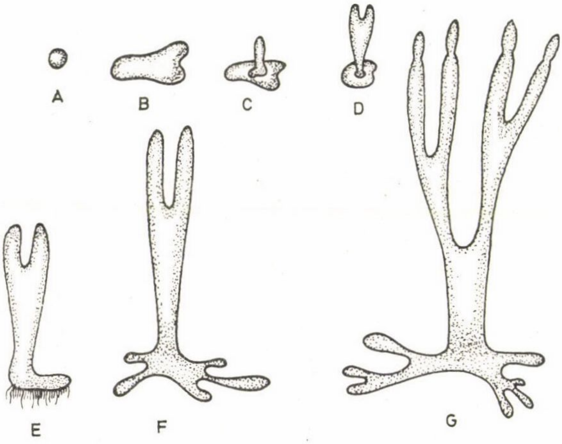

Fig. 3. Development of leaves from algae (leaf theory)

The presented theories are fairly well supported by the leaf types of classes and species belonging to the *Pteridophytae* (Fig. 4/A—G). The primitive shrubs had — and the *Psilotum* species have even today — spike-like leaves. There are already vascular bundles in the shoot axis but they do not branch off in the microphyllum. A somewhat larger but almost similarly spike-shaped microphyllum is found on the shoots of *Lycopodia*. By a growth in width the spike-like subulate leaves became lamelliform in the *Selaginellae*. In the *Equisitum* microphylla developing on different levels became planar during development, and the leaf edges grew together. Planation of telomes occurred in the fossile fern *Protopteridium* (Fig. 5). The further develop-

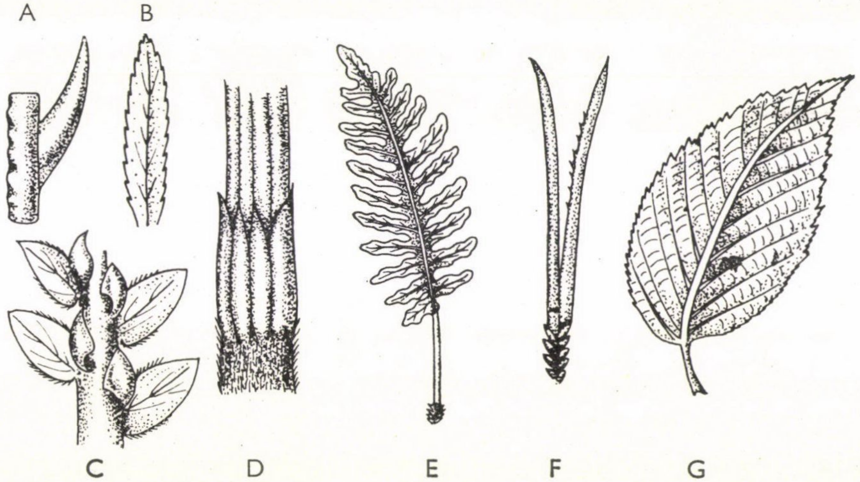

Fig. 4. Some stages of leaf development. *A Psilotum triquertum; B Lycopodium clavatum; C Selaginella Martensii; D Equisetum arvense; E Polypodium vulgare; E Pinus silvestris; G Fagus silvatica*

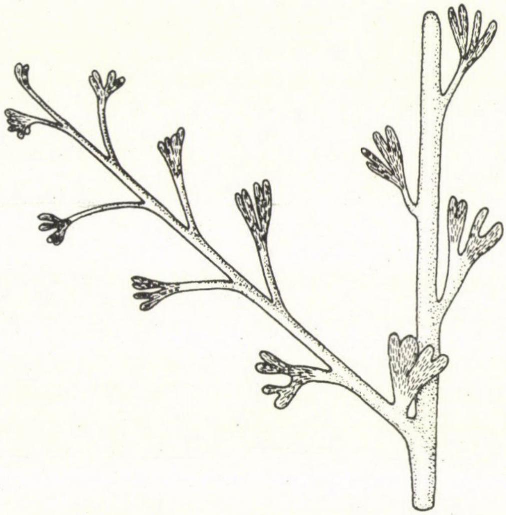

Fig. 5. Initial development phase of a *Protopteridium* leaf

ment of this leaf type can be observed in the ferns. From the pinnated leaves of seedy ferns developed in one line of development the acerose leaf of *Gymnospermae*, and in the other line of development the bladed leaf of *Angiospermae*.

Definite stages of development can be observed in the histogenesis of leaves too, agreeing in almost every case in the leaf primordia differentiating on the side of the growing tip of the shoot. At a lower stage of organization (Fig. 6/A, A_1) the leaves are initiated from the outermost cell-row, the protodermic cell layer, in the following way; 2—3 cells above each other radially elongate and divide with walls parallel to the surface. The produced cells then become larger and appear on the side of the growing tip of the shoot as leaf primordia. The two outer rows of the three cell layers making up the leaf primordia form the protoderm of the developing leaf, and the inner cell-row will become the mesophyllum. The development of leaves in *Equisetum* and *Polypodiaceae* begins, on the other hand, with the formation and dividing of a single two-section leading cell, and the adjacent as well as the derived cells take part in the leaf formation as a-eristemic cell group.

On the growing tips of shoots in seedy plants leaf primordia are organized in a somewhat deeper layer. Their development begins with a periclinal dividing of cells in the subprotodermic layer under the protoderm, and the protodermic tissue follows the surface increase of the leaf primordium with anticlinal walls.

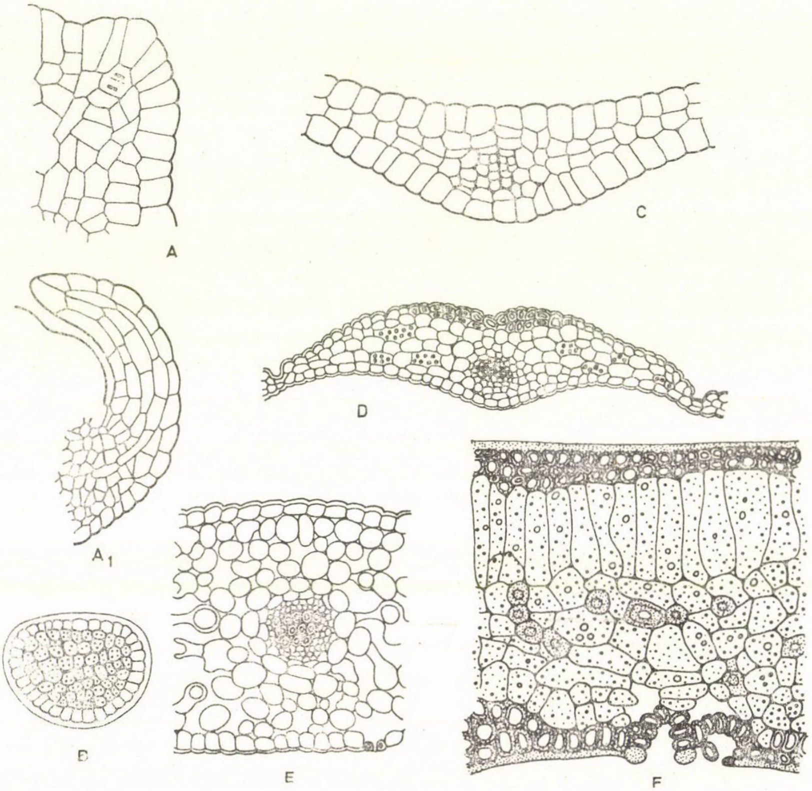

Fig. 6. **Histogenesis of leaves; A_1, A_2 *Psilotum triquetrum* leaf initiation: *B* km of the former: *C Selaginella martensii; D Equisetum arvense; E Dryopteris filix-mas; F Dioon edule***

The leaves of *Pteridophytae* are of apical growth. This is in relation to some extent with the continuous functioning of the leading cell. This process conspicuous in the *Polypodiaceae.* By continuously functioning the leading cell which has produced the leaf primordium increases the apical part and it is this way that the leaf develops in full length. The base of the leaf soon becomes stabilized, while the apical part maintains its meristemic character. During their development the leaves of ferns are rolled in protecting the sensitive growing tip. In these plants the lateral growth is much more considerable. Growth in width occurs through the bordering cells produced by the apical meristemic cells. In the case of the other type of longitudinal growth increase begins at the apical part of the leaf primordium, but the further growth is soon taken over by the basal part of the young leaf. This basal growth appears in *Welsitschia* and in the *Angiospermae.* The young leaf primordia are soon divided into two parts. The apical part of the leaf — as it has been mentioned — soon becomes stabilized while the basal meristem of the leaf primordium continues dividing. In its functioning this basal meristem may be divided into several phases; lamellar meristem and sagittal meristem. The lamellar meristem is the first to begin functioning. From the sagittal meristem first the leaf base then the petiole develops. Simultaneously with the longitudinal growth of leaf blades a considerable lateral growth also takes place through cell division in the submarginal meristems and the dividing cells of interostal zones. Along the leaf veins and in the petiole considerable thickening can be observed as a result of cell division in the epidermis and the intervenial tissue zones.

In the tissue structure of developed leaves of shooted plants an apparent sequence of development can be established (Fig. 6/B—F). The leaf of *Psilotum* is covered by a one-layer epidermis with no stomata on and no vascular bundles differentiating in it. Within the epidermis there is an assimilating ground tissue. Water is delivered to the site of assimilation from cell to cell. Organic matters are transported toward the base of the leaf then into the petiole in the same way. At a somewhat higher stage, similarly in the microphylla of *Lycopodium* and *Selaginella* some vascular bundles appear. In the slightly flattened leaf type a certain degree of lateral growth also occurs. Between the upper and lower epidermis the mesophyllum still has a homogeneous structure.

In the leaves of *Equisetums* tissue differentiation increases. Above the vascular bundle, next to the upper epidermis collenchyma develops. In the leaves of fens hydrocentric vascular bundles differentiate and in the mesophyllum tissue differentiation into palisade parenchyma and spongy parenchyma begins: this type of organization becomes expressed in the *Cycadae* and this bifacial heterogeneous structure is then characteristic of most *Angiospermae.*

*

Prepared at the Department of Applied Botany and Histogeny of the Eötvös Loránd University, Budapest.

P. Gracza

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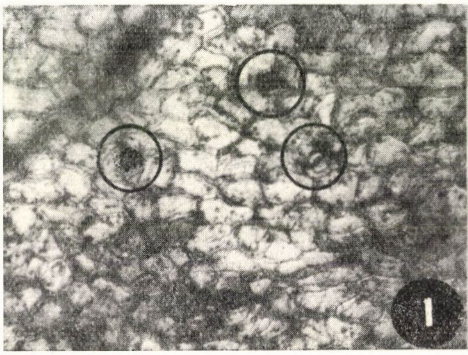
STUDIES ON THE SIZE AND DISTRIBUTION OF STOMATA IN JUTE (CORCHORUS OLITORIUS AND C. CAPSULARIS) AND ITS BEARING ON RESISTANCE TO DROUGHT

The importance of stomata in the control of the physiological mechanism of plants has received considerable attention since the time of Von Mohl, Schwender and others in the last century. In this century studies on the density and sizes of stomata and their relationship with carbon assimilation, transpiration and respiration in different species have been made by several investigators of whom a few may be mentioned (Brown—Escombe 1900, Eckerson 1908, Kolkunov 1905, Reed—Hirano 1931, Maximov 1929 and Carolin 1954). According to Salisbury (1927) stomatal size and density are greatly influenced by environment and their position on the plant. Kundu—Sen (1958) studied the development and structure of the foliage of some of the varieties of "Tossa" (*C. olitorius*) and "White" (*Corochorus capsularis*) jute.



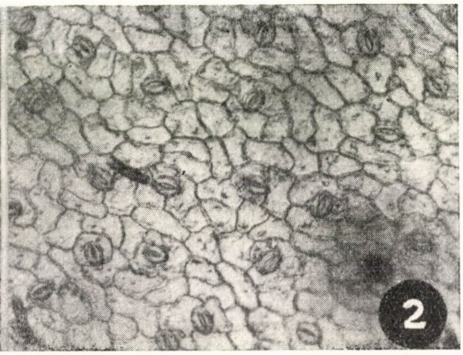


Fig. 1. Stomata on the upper epidermis of a leaf (shown within circles) in *C. olitorius*, var. CG
Fig. 2. Stomata on the upper epidermis of a leaf (shown within circles) in *C. capsularis* (Var. JRC—321)

In practical agriculture, in jute, similarly to other crops of economic value, studies on the efficacy of the foliar absorption of nutrients have received some attention as a means of obtaining higher yields (Saha—Goswami—Pramanik 1967, Karsaha 1963).

In view of the importance of the structure as well as of the number of the stomata in the performance of some of the vital processes of life in plants, the present study was undertaken to obtain some more information in the two cultivated species of jute.

Eight varieties, four from each species (*C. olitorius* and *C. capsularis*) were selected for the above study. The varieties differed in their yield potentiality, maturity and some other morphological features. One of the four varieties in each species had some wild characteristics, viz. short and much branched stem. The varieties were CG, JRO-630, JRO-632 and wild-olitorius and JRC-321, JRC-919, D154 and Kulkarni (Wild-nature) under *C. olitorius* and *C. capsularis* respectively. They were grown in the field and leaf-samples from five plants were taken at random from a crop of 100 days' old. Peels were taken from five different spots of each surface of a leaf, and fixed in 70% alcohol, and the length and breadth of 25 stomata were measured from each of the peeled epidermis by an ocular micrometer after standardizing with the stage micrometer.

The number of stomata per microscopic field which was afterwards converted to number per cm^2 of a leaf was counted. The area of a stoma was determined by multiplying its length

Table 1

Length of Stomata (in μ)

Species	Variety	Epidermis		Mean
		Upper	Lower	
C. olitorius	Wild-olitorius	59.66	46.46	53.06
	JRO-632	87.65	49.83	58.74
	JRC-620	51.48	46.86	49.17
	C.G.	64.02	53.79	58.90
	Species, mean (1)	60.702	49.235	54.967
C. capsularis	D-154	56.10	49.83	52.96
	Kulkarni	58.08	60.72	59.40
	JRC-919	57.42	53.46	55.44
	JRC-321	56.10	53.13	54.61
	Species, (mean (2)	56.925	54.285	55.602
Mean		58.81	51.76	55.28

1. C.D. for variety means (means over epidermis)
   at 5% P = 1.65 SE = 0.45
   at 1% P = 2.23
2. C.D. for epidermis means (means over varieties)
   at 5% P = 0.90 SE = 0.24
   at 1% P = 2.10
3. C.D. for epidermis means for same variety
   at 5% P = 1.78 SE = 0.68
4. C.D. for variety means for the same epidermis or different epidermis
   at 5% P = 2.46 SE = 0.65
   at 1% P = 3.30

and breadth by 0.7854 (a factor for an elliptical pore). The data were processed statistically and given in Tables 1—6.

Three different types of stomata were noted in the above varieties studied; (i) Ranunculaceous in which the stomatal apparatus is devoid of any true subsidiary cells, (ii) Rubiaceous in which there are one or two subsidiary cells which run parallel to the long axis of the pore and guard cells and (iii) Cruciferous in which the stoma is surrounded by three cells of which one is distinctly smaller than the other two and which is only found in a very small number in one variety JRC-919 belonging to *C. capsularis.* D154 is characterized by a higher frequency of the Ranunculaceous type. In other varieties of *C. capsularis* the Rubiaceous type was found more in number. Among the varieties of *C. olitorius*, CG was found to have Ranunculaceous and Rubiaceous type in more or less equal proportions, while in other varieties the Ranunculaceous type was slightly more in number than the Rubiaceous one.

Length and breadth of stomata. It was observed from the analysis of variance (Table 6) that the two species did not differ statistically in the lengths of their stomata but did so in respect of the breadths, while both the length and breadths of the stomata differed significantly

Table 2

Breadth of Stomata (in μ)

Species	Variety	Epidermis		Mean
		Upper	Lower	
C. olitorius	Wild-olitorius	44.68	31.35	38.01
	JRO-632	46.39	37.09	41.74
	JRO-620	39.00	40.78	39.89
	C.G.	50.16	42.10	46.13
	Species, mean (1)	45.06	37.83	41.44
C. capsularis	D-154	30.79	37.02	33.90
	Kulkarni	40.65	38.74	39.69
	JRC-919	41.91	40.26	41.08
	JRC-321	44.94	40.59	42.76
	Species, mean (2)	39.57	39.15	39.36
Mean		42.31	38.49	40.40

1. C.D. for variety means (means over epidermis)
at 5% P = 2.28 SE = 1.09
at 1% P = 5.31
2. C.D. for epidermis means (means over varieties)
at 5% P = 1.03 SE = 0.49
at 1% P = 1.39
3. C.D. for epidermis means for same variety
at 5% P = 2.96 SE = 1.45
at 1% P = 3.99
4. C.D. for variety means for the same epidermis for different epidermis
at 5% P = 3.10 SE = 1.52
at 1% P = 4.19

at 05 and 01 probabilities among the different varieties within each species. In D154, the breadths of the stomata in the lower epidermis were conspicuously more than those on the upper epidermis of JRO-620, and the lengths in Kulkarni on the lower epidermis were slightly more than those found on the upper epidermis. The lengths in JRO-632 and CG were of the same order which differed significantly from Wild-olitorius and JRO-620, and the last one exhibited the minimum which again differed significantly from Wild-olitorius (Table 1).

As regards to breadths, however, there was no significant difference between Wild-olitorius and JRO-620 which exhibited to minimum breadth, but CG and JRO-632 differed significantly, CG being characterized by the broadest stomata (Table 2).

Stomatal area. The area of a stoma was found to vary significantly between the species as well as amongst the varieties, within each species. In general, significantly smaller stomata were noted on the lower epidermis of all the varieties studied. With the exception of JRO-620 the differences in size of the stomata were more pronounced in the varieties of *C. olitorius* than among the varieties of *C. capsularis.* In general, *C. capsularis* is characterized by comparatively smaller sizes of stomata than *C. olitorius.* CG and JRO-632 are characterized by

Table 3

Length and breadth ratio of Stomata

Species	Variety	Epidermis		Mean
		Upper	Lower	
C. olitorius	Wild-olitorius	1.34	1.42	1.38
	JRO-632	1.36	1.30	1.33
	JRO-620	1.31	1.14	1.22
	C.G.	1.27	1.26	1.26
	Species, mean (1)	1.32	1.28	1.29
C. capsularis	D-154	1.40	1.34	1.37
	Kulkarni	1.12	1.57	1.34
	JRC-919	1.37	1.32	1.34
	JRC-321	1.24	1.30	1.27
	Species, mean (2)	1.28	1.38	1.33
Mean		1.30	1.33	1.31

1. C.D. for variety means (means over epidermis)
at 5% P = 0.077 SE = .038
at 1% P = 0.104
2. C.D. for epidermis means (means over varieties); not significant.
3. C.D. for epidermis means for the same variety
at 5% P = 0.92 SE = .045
at 1% P = 0.123
4. C.D. for variety means for the same epidermis or different epidermis
at 5% P = 0.098 SE = .048
at 1% P = 0.132

bigger stomata amongst the *C. olitorius* varieties but having no statistical difference between them, in contrast to the smaller stomata in JRO-620 and Wild-olitorius which again differed significantly from those of both CG and JRO-632. JRO-620 and Wild-olitorius were found to fall in the same group statistically. The variety D154 had the smallest stomata which differed significantly from those of Kulkarni, JRC-919 and JRC-321 and the latter three varieties did not exhibit any statistical difference in respect of stomatal area (Table 3).

Stomatal frequency. The number of stomata per square centimetre of a leaf was found to be significantly higher in *C. olitorius* than in *C. capsularis.* In all the varieties the frequency was more on the lower surface than on the upper surface, again the ratio of frequency on the upper and lower surface was found to be approximately 1 : 3 and 1 : 2 in *C. olitorius* and *C. capsularis* respectively. The varietal differences in respect of this characteristic within *C. olitorius* were statistically significant at both .05 and .01 probabilities while in *C. capsularis* it was significant only at .05 P. The frequency was found lowest in Wild-olitorius although it had no significant difference with JRO-632 and no significant difference was observed between CG and JRO-632. Of all the varieties, studied above, JRO-620 exhibited maximum frequency and the next in order were JRC-321, CG, Kulkarni, JRO-632, Wild-olitorius, JRC-919 and the lowest was D154 which had more or less the same frequency as JRC-919 (Table 4).

Length and breadth ratio of stomata. Differences in the ratio of length to breadth of the

Table 4

Number of Stomata per cm² of a leaf

Species	Variety	Epidermis		Mean
		Upper	Lower	
C. olitorius	Wild-olitorius	1445.54	3684.03	2564.78
	JRO-632	1424.69	3968.93	2697.33
	JRO-620	1352.56	5333.07	3352.81
	C.G.	1410.45	4313.82	2862.13
	Species, mean (1)	1408.31	4325.32	2866.76
C. capsularis	D-154	1452.56	3620.81	2536.68
	Kulkarni	1719.21	3778.76	2748.98
	JRC-919	1463.08	3590.05	2526.56
	JRC-321	1938.66	4431.36	3185.01
	Species, mean (2)	1643.38	3855.24	2749.31
Mean		1525.84	4090.23	2808.03

1. C.D. for variety means (means over epidermis)
   at 5% P = 236.29 SE = 164.90
   at 1% P = 453.48
2. C.D. for epidermis means (means over varieties)
   at 5% P = 128.76 SE = 63.15
   at 1% P = 173.67
3. C.D. for epidermis means for the same variety
   at 5% P = 379.27 SE = 184.20
   at 1% P = 511.37
4. C.D. for variety means for the same epidermis or different epidermis
   at 5% P = 428.57 SE = 208.76
   at 1% P = 577.86

stomata between species and amongst the varieties within the species were found to be statistically significant. This ratio, however, was the same in the two epidermis, irrespective of the species. L/B ratio was found a little higher in *C. capsularis*. Amongst all the varieties studied JRO-620 exhibited the lowest L/B with CG, and JRC-321 in the same statistical group, while Wild-olitorius was marked by the highest L/B with JRO-632 and D154, Kulkarni and JRC-919 in the same group (Table 5).

Our observations on the three types of stomata which are present in the above varieties of jute are in agreement with the findings of Kundu—Sen (1958). Again, the distribution of the stomata on the two surfaces of the leaves in the varieties of *C. olitorius* is characterized by having a greater number on the lower surface as compared to those in *C. capsularis*. This also confirms the views of Kundu—Sen (1958) who reported that the ratio of the distribution of the stomata on the upper to the lower surface varies from 1 : 1 to 1 : 2 in *C. capsularis* and 1 : 2 to 1 : 3 in *C. olitorius* respectively.

Smaller and less number stomata per cm² is, in general, a characteristic of *C. capsularis* of which the variety D154 has the smallest in size and minimum in frequency, although another variety JRC-919 falls in the same statistical group when only the frequency is con-

Table 5

Stomatal area (square μ)

Species	Variety	Epidermis		Mean
		Upper	Lower	
C. olitorius	Wild-olitorius	635.84	346.80	491.80
	JRO-632	791.80	440.25	616.01
	JRO-820	478.37	456.75	467.54
	C.G.	766.59	540.01	653.30
		668.15	445.92	
C. capsularis	D-154	533.05	440.35	486.95
	Kulkarni	562.45	560.24	561.33
	JRC-919	574.13	513.38	543.74
	JRC-321	601.62	513.94	557.76
Mean		567.80	507.08	

1. C.D. for variety means (means over epidermis)
   at 5% P = 54.32 SE = 26.63
   at 1% P = 73.23
2. C.D. for epidermis means (means over varieties)
   at 5% P = 5.64 SE = 2.77
   at 1% P = 7.62
3. C.D. for epidermis means for the same variety
   at 5% P = 16.00 SE = 7.85
4. C.D. for variety means for the same epidermis or different epidermis
   at 5% P = 55.51 SE = 27.19
   at 1% P = 74.84

sidered. Kolkunov (1905) reported that the drought resistant varieties of wheat have smaller stomata in comparison to drought susceptible ones. This suggests, the variety D154 naturally has the maximum efficiency to resist drought, although, in general, most of the *C. capsularis* varieties have a capacity to resist drought to a considerable extent. In practice, probably because of photo insensitiveness the *C. capsularis* varieties are usually sown early, before the monsoon sets in and sometimes have to survive severe drought conditions.

Kundu—Sen (1958) have observed the lowest number of stomata in JRC-5854, which is followed by JRC-206 and D154 amongst the varieties of *C. capsularis*. However, stomatal size and density are highly influenced by environment and their position on the plant (Salisbury 1927). The variety JRC-206, though not included in our study, has some similarities with JRC-919, respecting its time of maturity, growth, pigmentation pattern and stomatal characteristics and it falls in the same group as D154. It has also been recorded (Kundu *et al.* 1959) that both JRC-919 and JRC-206 have a common origin, i.e., from an exotic material. The occurrence of the lowest number of stomata in JRC-5854 may be due to reasons connected with its origin. It is reported to be a hybrid, one of the parents being D154 (Joseph 1969). However, in our observations the variety D154 showed the lowest frequency.

Amongst *C. olitorius* varieties the lowest frequency was observed in Wild-olitorius and the frequency of CG came after JRO-632 although there was no significant difference between

Table 6

Analysis of variance

Source	DF	Mean sum of squares				
		Length	Breadth	L/B.	No. of stomata	Area of stomata
1. Species	1	2.24	10.06**	0.10**	2 298.13*	2 327.32
2. Varieties within *C. olitorius*	3	67.78**	38.71**	0.043*	6 576.87**	25 263.58**
3. Varieties within *C. capsularis*	3	21.98**	10.50**	0.086**	2 912.08**	3 616.17*
4. Error (a) Within varieties among plants	32	1.02	1.90	0.0075	792.94	1 083.03
5. Epidermis	1	303.66**	166.06**	0.002	764 297.63**	121 281.67**
6. Epi×Var	7	31.94*	21.15**	0.024*	5 780.42**	12 874.19**
7. Error (b)	32	1.21	1.61	0.0052	496.47	47.29

* Significant at 5%
** Significant at 1%

the two. However, the lowest frequency was observed by KUNDU—SEN (1958) in CG, though of course, Wild-olitorius was not included in their studies. We observed that Wild-olitorius and JRO-620 have stomata which are significantly smaller in size as compared to CG and JRO-632, which characteristic is considered important in withstanding drought to a great extent as indicated by KOLKUNOV (1905).

In jute, at a lower level of fertilizer (urea) treatment, by spray, the increase in fibre yield over the control was relatively more in *C. olitorius* than *C. capsularis* (SAHA *et al.* 1967).

This seems to be due to the larger size and higher frequency of stomata in *C. olitorius* (JRO-632). Before passing such comments, however, the nature and thickness of the cuticle should also be taken into consideration. KUNDU—SEN (1958) have stressed the point that the distribution of the stomata and the thickness of the cuticle are of interest from the point of view of total or partial resistance to drought.

From the above results it would appear reasonable to think that the size and distribution of the stomata play an important role in protecting plants against drought, and they are specific in nature, although there is much environmental stress acting on the development of the stomata, yet it is also true that the ability of plants within a variety or species to be modified by the environment is genetically determined. However, the possibility of the plant withstanding drought by means of various physiological and anatomical adjustments makes it insufficient to determine the degree of drought resistance on the basis of a single feature, like the smaller size of cells, even if it is a feature of major importance (MAXIMOV 1929).

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G. C. MITRA, N. C. BASU

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THE GERMINATION MECHANISM AND ITS ECOLOGICAL SIGNIFICANCE IN THE WEED DIGERA ALTERNIFOLIA ASCHERS

Digera alternifolia Aschers Syn. *D. arvensis* Linn. is one of the common weeds of the crop fields of the country. The seeds ordinarily fail to germinate in a laboratory. Seeds kept under different temperatures for different durations and alterations, failed to germinate. To achieve germination different storage conditions (as dry moist storage at various temperatures in open and closed containers, also with lime, soil, calcium chloride, burial in soil at various depths, water logging etc.), different pretreatments (as with acids, alkalies, alcohol, glycerine, hydrogen peroxide, carbon tetrachloride for various durations, solutions of carbonates, nitrates, chlorides, urea, thiourea, gibberlic acid, etc.) and moistening agents (as soil extract, 0.2% solution of nitrates, lime water etc.) were employed. But in most of the conditions either the seed failed to germinate in the first three or four months or germinated to an extent of less than 10% (Dubey 1967).

In fact a great number of treatments and pretreatments separately and in combinations were tried to get a maximum percentage of germination in a minimum span of time in the laboratory. The described conditions and pretreatments in their particular combinations were found suitable and can well be compared with possibly existing conditions in nature.

Certain mechanisms for germination have been described by Barton (1936), Mall (1954), Koller (1955), Koller—Negbi (1955), and Rees (1962). The effect of several factors in germination are studied by Steinbauer—Grigsby (1957) and Davis—McCarty (1966). The role of promoting substances from crop root exudates is shown by Sunderland (1960) and Kust (1966). But the combination of many factors required for germination by this seed appears to be unusual and hence the details of the study are given below. The study presents the role of certain factors in germination of more and more seeds of the weed in a shorter and shorter period under laboratory conditions.

Seed collection. Nuts, hereafter called seeds (AMAN 1964), were collected at various time intervals from the crop field of Ujjain and other parts of the country. Seeds were stored in glass bottles.

Germination. Seeds were placed for germination on double layers of moist filter paper in petridishes. A seed was considered to be germinated by the emergence of the radicle.

Experiment 1. Germination of seeds at room temperature and at 15°C. The seeds collected on 15th October, 1st November and 15th November of 1965 were placed for germination, on the day after collection. No seed could germinate in the first 8 months though they were viable.

Experiment 2. Germination of seeds at 35°C. The seeds, collected on the dates described in Experiment 1, were kept for germination at 35 ± 1°C. In 10 months time the germination was less than 10% irrespective of the date of collection. But this condition appeared to be better at room temperature and 15°C. It was found that in the first 3 months no seed could germinate (Table 1).

Experiment 3. Effect of duration and temperature during dry storage. Glass bottles containing seeds were kept for various months at 15°C, 35°C and 45°C (for 24 hours in each temperature), room temperature and at an alternation of 35°C to 15°C for 18/6 hours, respectively. Seeds from these bottles were kept for germination at 35°C ± 1.2°C. The most suitable condition for better germination appeared to be the 45°C temperature and storage at this temperature for about 6—8 months. Longer storage appeared to be injurious (Table 2).

Table 1

Germination of seeds at 35°C

Date of collection	Percentage of germination in the months										Total
	1	2	3	4	5	6	7	8	9	10	
15th October, 1965	—	—	—	1	—	—	2	—	2	4	9
1st November, 1965	—	—	—	1	1	—	2	—	1	1	6
15th November, 1965	—	—	—	1	1	1	—	4	1	—	8

Table 2

Effect of storage durations and temperature during storage on germination

Storage temperature °C	% of seeds germinated					
	Storage durations in months					
	2	4	6	8	10	12
15 ± .6	—	—	1	5	8	8
35 ± 15 ± .6 (18 hr. resp.)	—	1	1	7	12	13
35 ± 1	1	1	4	8	14	17
45 ± 1.7 °C	3	10	20	24	17	12
Lab. Temp. 28 ± 1.4° (control)	—	—	2	5	5	12

F for temperature, duration and interaction significant at 0.001 level

Table 3

Effect of washing on germination

Washing interval	Percentage germination in months					Total %
	1	2	3	4	5	
Daily	6	9	10	6	1	32
Weekly	4	4	7	5	2	22
Monthly	3	4	2	4	1	14
Control (no washing)	1	0	2	1	5	9

F for washing significant at 0.01 level

Table 4

Effect of acid scarification on germination in addition to preceding treatments

Acid scarification (Time in minutes)	Percentage in fortnights								Total %
	1	2	3	4	5	6	7	8	
15	1	1	4	3	1	3	1	—	14
30	1	2	5	7	2	5	1	—	23
45	3	7	8	5	7	4	2	1	37
60	2	1	5	8	7	2	—	—	25
Control (No washing and no scarification)	—	—	1	1	4	1	—	—	7

F for scarification and time significant at 0.001 level

Experiment 4. The effect of washing the petridishes containing seeds for germination. Noting the effect of duration and temperature, seeds utilized in all further experiments were stored at 45°C ± 1.7°C temperature for 7 months. Such pretreated seeds were kept for germination at 35 ± 1.2°C and the petridishes were washed daily, weekly and monthly with tap water. Washing had a significant effect because daily washing resulted in 31% of the seeds germinating in the 4 months period. In the 5th month and onward the germination was low and nil respectively (Table 3).

Experiment 5. Effect of scarification on pretreated seeds with daily washing. Seeds stored for 7 months at 45 ± 1.7°C were scarified with conc. sulphuric acid for various durations (in minutes). The petridishes containing seeds were kept at 35 ± 1.2°C and were washed daily. Acid scarification for 45 minutes proved helpful in increasing the percentage of germination to 34 in three months. In later months the germination was very poor, even in the 4th month it increased by 3%. Also, a shorter or longer scarification time than 45 minutes had either a reduced or an injurious effect on the seeds (Table 4).

Experiment 6. Effect of crop root exudate in germination. Seeds stored at 45 ± 1.7°C for 7 months, scarified for 45 minutes and thoroughly leached, were soaked in a solution of promoter for 4 hours. These seeds were kept for germination at 35 ± 1.2°C. The promoter was used to moisten the filter paper instead of water. Different grades of the promoter were made and used.

Promoter solution was prepared by putting 100 germinating seeds of *Sorghum vulgare* Pers. for 5 days, in the petridishes containing 10 ml water. This volume was always made up to 10 ml during these 5 days by subsequently adding the required water. The decanted liquid was named promotor solution. The promoting effects of root exudates are significant, as within 2 months time the germination percentage moved to 60 (when the undiluted solution of promoter was used). The effect of promoter was much reduced when it was diluted to 100 times (Table 5).

Digera alternifolia Aschers is a well-known weed of the crop fields of the country. A three and a half years study of germination revealed interesting mechanisms which are of "immense ecological significance in the life cycle of such successful weeds" (HARPER 1957).

Table 5

Effect of promoter on germination of pretreated seeds

Dilution of promoter	% germination in two months
1 : 0	60
1 : 10	39
1 : 100	27
No promoter	22

The observation that the seeds in the first 3—4 months fail to germinate but start germination after this period clearly points towards the after ripening of the seed. Storage of the seeds at higher temperature (45 ± 1.7°C) and the resultant increases in germination lead to the conclusion that temperature enhanced the after ripening processes and in addition had a weathering effect on the seed (fruit) coat. A similar condition of temperature is available in nature during March to July when the temperature ranges between 35 to 44°C. The significant effect of leaching shows the presence of some inhibitors in the seed. The scarification only affects the increase in the inhibition of water by the coat, which in nature is possibly met with the weathering caused by higher temperature and also by the microbial activity. Further, the increased percentage of germination of such pretreated seeds in the presence of the crop root exudate will only mean that promoters are also necessary for enhancing the processes of the embryo to become a seedling.

Henceforth it becomes evident that the seed expresses an embryonal dormancy, a seed coat dormancy, inhibitors in the body and needs promoting substances too. A successful germination of the weed seed thus requires various conditions and is rather a case of multi-factorial germination. In fact lack of any of such pretreatments can lengthen the period of germination and can make the later intermittent. All these conditions or factors in nature are comparable with the naturally borne treatments as rest period, summer temperature, rainfall and the root exudates from the crop.

A combination of all such conditions safely exist in the crop fields which thus become an ideal habitat for the weeds establishment. One interesting feature was noted that all these required treatments, which were given in a laboratory if applied to fresh seeds in the first 3—4 months, did not have much effect on the seed or the seed behaved indifferently to these conditions in this period and completely failed to germinate.

All these factors, ecologically speaking can converge to a word; adaptation. The reason for this is that when the fate of a weed is controlled by a definite need of restricted ecological conditions it is then bound to adjust and adapt (MALL 1954). Indeed if all the conditions are seen in correlation in the life cycle of the weed, they will prove to be adjustment bundles instead of ecological hurdles (BILLINGS 1957). The validity of this statement can be understood by the following description.

The incapability of a fresh seed to germinate is nothing but an escape from the damage to itself, because by the time it is shed (October—November) from the plant, the moisture in the soil remains inadequate for leaching out the inhibitors. Henceforth the weed seed undergoes a period of rest during which the after ripening takes place. By this time, the summer (April—June) starts and higher temperatures of the season are utilized for enhancing the after ripening and weathering of the coat as well. At the end of June the rain starts which will enable the leaching of inhibitors and will supply enough moisture to the crop seed to germinate. So, while the seed is preparing for germination, the crop seedlings will come up releasing the exudate from the roots and helping the weed seed to germinate and grow better.

This is a great physioecological achievement of this weed as it utilizes the crop material to grow and to damage the former in the later days. This physiological set-up of *Digera alternifolia*, is a baffling problem for the farmers who leave the fields abandoned for a year with the hope that all the seeds of the weed will come up and they can uproot the seedlings, but as the above accounts show, their hope remains unfulfilled. It is thus worth stating that every step of the development of the weed's seed and its germination is a well adapted ecological stage, and the weed has moved to a high level of ecological attainment.

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Prepared at the Botany Department, University of Jodhpur, Jodphur; School of Studies in Botany, Vikram University, Ujjain.

P. S. DUBEY, L. P. MALL

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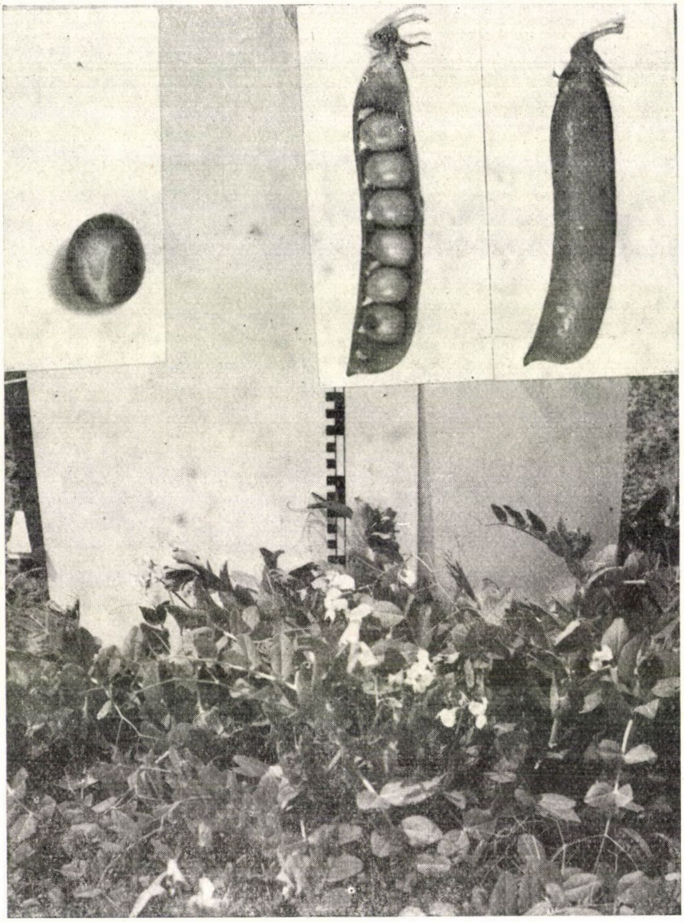

FODDER PEA "IREGI SÁRGA"

(Synonyms; Iregi P. 1., I. P. 1)

Taxonomical place: Pisum sativum L. convar. *vulgare* Alef. var. *episcopi* Alef.

Origin: Edelperle × Express.

Beginning of breeding: 1947, Iregszemcse.

State qualification: state certified, improved variety, 1963: first accepted 1959.

Breeder: Prof. Dr Ernő Kurnik, Iregszemcse.

General characterization: good yielding ability; as for seed production it is superior to the Hungarian husking and fodder peas but is exceeded by some foreign varieties; its early seed ripening is a characteristic it surpasses all other varieties by; it is a hardy fodder pea of good storability which can be harvested mechanically (Mrs. Iványi 1962, Kapás *et al.* 1965).

Morphological description:

Root system: penetrates deep into the soil.

Shoot system: thickly branched, tough.

Stem: 45—50 cm high, its internodes are short, therefore the leaves are thickly set: its colour is medium green.

Foliage: the leaves are relatively small, of greyish green colour, glaucous surface, turning into a moderately developed tendril. The leaflets are mostly set in pairs, their shape is ellyptical, the apex is cut with fine hairs on it, the edges are unbroken, and a very short leaflet-peduncle is attached to the rounded base. The stipule is half-egg shaped, medium green and slightly spotted.

Flowers: are set on the floral axis in pairs: the corolla is white, the standard large, of flat semi-circular shape, with a sharp tooth on the apex; the edges are crisped and the bases V-shaped; the wing-blades are heart-shaped.

Legume: 6—13 per plant; 5—7.5 cm long, pale green colour, straight (occasionally slightly bent), narrow, with pointed tips. The pods contain 6—8 seeds each.

Seed: pale green when unripe and greenish yellow when ripe; small, smooth surfaced, irregularly round (therefore unsuitable for husking; Mrs. Iványi 1962). Thousand-seed-weight: 200—220 g (Kapás *et al.* 1965).

Biological character:

Vegetation period: from sprouting to flowering 38—50 days are required, while from flowering to ripening the development takes 36—42 days; the total vegetation period extends to 80—86 days (with seeds sown in the first half of April). The seeds ripen early, at the end of June, beginning of July (Komjáti 1959). Ripening takes place at the same time.

Water requirement: nothing special.

Resistance to diseases: it is a hardly variety avoiding most pests due to its earliness.

Farm technology requirement:

Seeding: end of March, beginning of April.

Soil requirement: nothing special, it can be equally grown in looser or more compact soils.

Productivity: seed production of several years average: 20.4 q/ha (fluctuation: 17.9—24.4 q/ha): percentage crude protein content to dry matter: 17.9—20.5% (Mrs Iványi 1962).

Region of cultivation: it can be grown all over Hungary.

*

Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen.

Gy. Mándy

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FORUM

EXPERIMENTS WITH THE "HORSE-RACE METHOD" OR OTHERWISE?

A generally accepted method of field experimentation is to include more than one variety or treatment simultaneously in a well designed experiment. By this identical conditions are created for the varieties or treatments with a view to make the comparison on reliable bases. We can rightly compare this method of field experimentation to a "horse-race" in which the start (in our case the beginning or setting up of the experiment) takes place in the same way. Only one of the variants of the experiment will give the best result similarly to the horse landing. If the experiments are repeated over several years often the variety or treatment outstanding in the first year will never be first again (Table 1). There are cases when in a three years experiment the same variety gives the best result twice, a three times repetition, however, is only possible in cases when there are very great differences between the varieties (which — on the other hand — distorts the experiment) for genuine genotypic reasons (Fig. 1).

Table 1

Seed yield of pea varieties, q/ha
(After Komjáti—Molnár 1969)

Variety	1965	1966	1967
Juwel	*41.7*	27.3	44.1
Mignon	41.2	24.8	49.3
Győztes	40.7	24.3	50.4
Mingomark	40.5	23.5	41.9
Perfection Dark Skinned	39.3	33.0	*53.7*
Debreceni	38.1	28.1	49.2
Lincoln	38.1	27.1	32.1
Chrestensens Cornel	25.9	*36.3*	44.8
Rajnai törpe	36.3	17.5	49.7
Konzervgyöngye	36.8	20.9	36.5

The above phenomenon is regularly observed in variety trials, its reason, however, is not searched for. When looking at the phenomenon from an ecological aspect it is easier to find an explanation for it. We have to start from the fact that every variety (stand) has

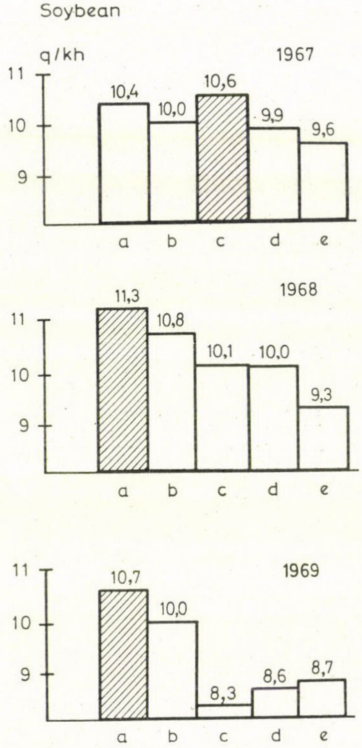

Fig. 1. Yield results of soybean varieties in a three years experiment. The vertical axis shows the yield amounts, q/cad. yoke; the horizontal one the varieties (columns); *a*: G. SZ. 3, *b*: Tápláni fodder soybean, *c*: Iregi Szürkebarát, *d*: Pannonia 10, *e*: Adepta (BAKOS 1971)

peculiar ecological requirements. If the variety is placed under conditions meeting its requirements it will give the highest result, while in the opposite case the latter will decrease. The same phenomenon is experienced if the crop results are studied in a succession of sowings. Such precise experiments were performed by KOLTAY (1971) over three years with wheat varieties (Table 2). The results showed that from the early time of sowing to the late one the yield values first gradually increased then slowly decreased. The change of values is similar to the course of the optimum curve (and that is what it actually is). Now, if we choose a sowing time (say that of 14th October) of the succession of sowing periods, none of the years examined will produce the highest yields of the varieties, only less than that in each case. This way no conclusion can be drawn concerning the maximum yields of the varieties. If we compare the yields of the two varieties in each year we see that Besostaya 1 gave higher yields in two years (1965, 1966) than Fertődi 293, but in 1967 the latter yielded more. When considering the averages we find that the yield of Fertődi 293 was higher with most of the sowing times. Thus, in possession of such a series the variety can be judged much more precisely than when studied with the above mentioned "horse-race method". Koltay's experimental data are interesting all the more so as he strictly kept the sowing dates each crop year. If the sowing times are changed from year to year (which is the usual practice) the fluctuations and differences will probably be even more conspicuous. We give the following data of Table 2 as an example:

	7 October 1965	14 October 1966	30 September 1967
	yield amount, kg/ha		
Fertődi 293	4800	5198	5407
Besostaya 1	3977	5626	5188
Difference	+823	−428	+219

Table 2

Yield amounts in a sowing time experiment with wheat warieties, kg/ha
(after Koltay 1971)

Sowing time	Fertődi 293				Besostaya 1			
	1965	1966	1967	Mean	1965	1966	1967	Mean
23 September	4274	5278	5466	5006	3641	5320	4552	4504
30 September	4711	5198	5407	5105	4004	5685	5188	4959
7 October	4800	5275	5518	5197	3977	6060	5397	5144
14 October	3641	5198	5386	4741	3660	5626	5129	4805
21 October	3803	5094	5077	4658	3571	5536	4191	4432
28 October	2280	4816	4458	3851	2799	5574	3867	4076
4 November	2058	4747	3753	3519	2405	5278	3746	3809
11 November	2428	4319	3624	3457	2641	5070	2658	3456
18 November	1618	—	2071	1844	2791	—	1146	1468
$SD_{5\%}$	356	284	316	318	390	257	476	374

Clearly, the value of the varieties can hardly be estimated if we do not know any other data on the productivity. In fact, those conducting the variety trials pay little attention to the date of sowing saying that the weather conditions are different anyway.

Whether or not we keep the dates of sowing the results show that in varieties showing no great differences in productivity the yields, though fluctuating from year to year, do not to differ distinctly from each other.

The question arises why the values of successive sowings change similarly to those of the optimum curve, and how this course could in essentials be explained (Mándy 1967). On the basis of repeated investigations made with plants sown in autumn and spring respectively, and even with perennial plants we can establish that in the case of experiments performed with successive sowing increasing or decreasing series of meteorological values are formed. In these series the intervals are not always the same, but they all change consequently in a certain direction. Our sunflower experiment in 1967 is a good example of this; here (Fig. 2) the maximum height of plants gradually increased in the sowing sequence parallel to the increase of mean temperature (broken line) during the vegetative period. At the same time the temperature and precipitation values (represented by wider and narrower columns) gradually decreased.

The above experiment on successive sowing pointed out that the sunflower is more sensitive to temperature than to precipitation. Still, according to the crop production handbooks the sunflower is a plant of "high water requirements" (Láng *et al.* 1970). This conclusion would hardly have been drawn if the "horse-race method" had been applied.

It is clear from what have been told above that by successive sowing we create an "ecological series" for our cultivated plants so that the effect of the changing values of the meteorological factors can be studied under identical soil conditions. If we think it over, the different sowing periods can be regarded as special ecologies, or — in a broader sense — even different "crop years". In this sense the ecological experiment performed with the sunflower varieties may correspond to a five years experiment series.

Although the distributed effect and regularity of the meteorological factors is evident in the case of the yield amount and plant height too, still the ecological effect is better demonstrated by the distribution of the phenomena of development within the stand. In whatever stand, this only requires an individual registration of the beginning — and possibly the termination — of the development phenomena. During the survey we shall find that there are plants in the stand which are quick to display their development phenomena, i.e. are of a more rapid growth than the other plants. Other plants, however, are too slow in their development. The interval between the early and late plants expresses the ecological reaction of the stand. The greater the interval, the more unfavourable the conditions under which the stand has developed, and vice versa. This way the reaction of the stand to the ecological conditions can be precisely measured. To the interval found in relation with the phenological phenomena we gave the name "individual amplitude" (MÁNDY 1970). A recently given individual amplitude

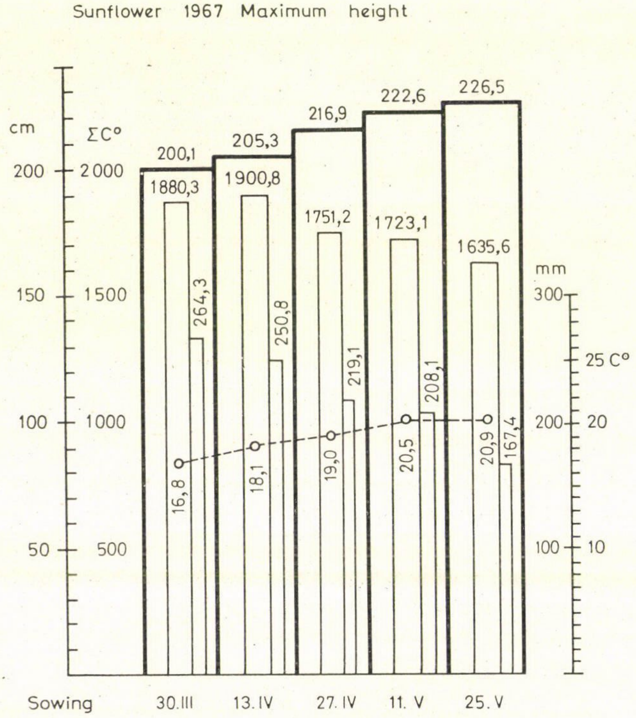

Fig. 2. Average height maxima of varieties in a sunflower experiment (wide, thickly outlined columns), and meteorological data: heat amount (ΣC°, medium wide, thinly outlined columns), mean temperature (indicated by small circles and a broken line), amount of precipitation (narrow, thinly outlined columns); the horizontal axis shows the sowing times, the vertical the meteorological and plant height values (cm)

clearly shows e.g. the differences in the ecological character of the varieties (Fig. 3). The individual amplitudes of waxen ripeness in 15 winter barley varieties show that beside the early varieties (Stavropolski, U 259, Engelens Dea, H. 55, Probsdorfer) there are late ones too (Odessa 17, Lédeci Beta, Tschermak, Jutta, Brucker, Bánkuti 14). Earliness means that sowing can be performed early, optimally in the second half of September, while the late varieties are best sown at the end of October.

We do not by any means say that an ecological setting (sowing) meeting the demands of the varieties only indicates their development properties. The slight fluctuation of phenological features is in relation with the different characteristics of the stand, first of all its productivity. This is clearly shown by the result of the winter wheat experiment. The lowest amplitude value of waxen ripeness corresponds to the highest values of grain and spicelet number per ear (Fig. 4).

The good stand development shows a close correlation with the quality as well, as shown by the result of our ecological experiment performed with poppy varieties (Table 3). Values obtained on the basis of the averages of varieties show that the amplitude minimum of

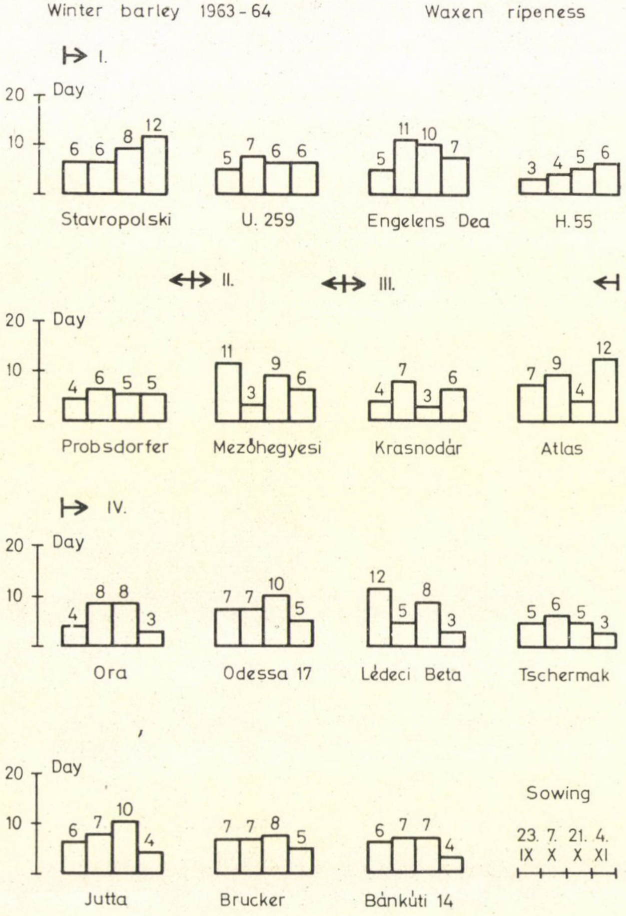

Fig. 3. **Individual amplitudes of waxen ripeness in winter barley varieties, in days**

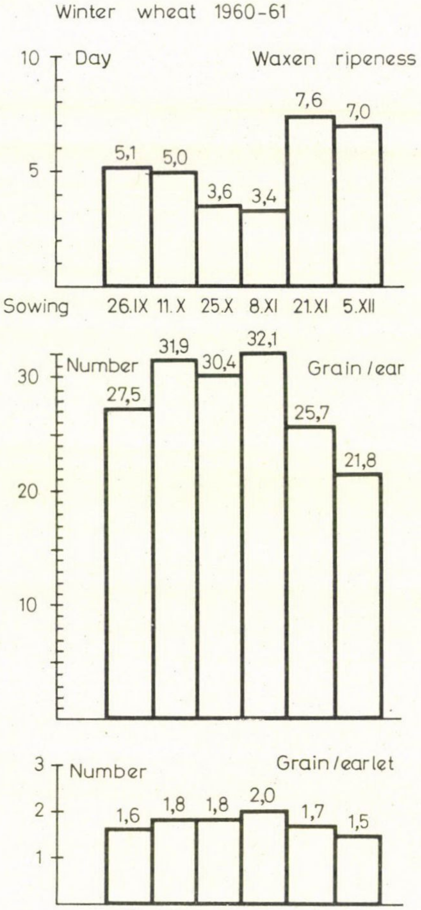

Fig. 4. Average individual amplitudes of waxen ripeness in winter wheat varieties, and mean values of grain per ear and grain per spicelet.

Table 3

Mean values of a poppy experiment (1967)
(MÁNDY 1969, unpublished data of a lecture)

Characteristics	Values with sowing performed on				
	28 February	14 March	28 March	11 April	25 April
Individual amplitude of the beginning of flowering, days	6.7	5.2	*5.0*	5.3	7.5
Morphine per mille	4.98	5.29	*5.43*	4.01	4.21
Oil content %	45.5	44.7	*46.2*	44.0	43.9

the beginning of flowering, the higher morphine and oil content fall within the same sowing period.

It is clearly seen from the above that in the case of investigations made with different varieties or treatments, a single date of sowing used in the experiment may give quite misleading results. In any variety trial or fertilization, metabolism, plant breeding or even plant protection experiment etc. it is desirable to give up the "horse-race method" and study the effects in an ecological experiment. This way we can avoid obtaining faulty results.

Gy. Mándy
University of Agrarian Sciences,
Department of Botany,
H—4001, Debrecen

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CONTRIBUTIONS TO THE PAPER OF E. TYIHÁK, A. PATTHY: "ON THE CHEMICAL NATURE OF 'PROMINE' AND 'RETINE' " PUBLISHED IN THIS PERIODICAL, *22*, (3–4)

WHAT IS THE CHEMICAL NATURE OF PROMINE AND RETINE?

There is no doubt that the two authors did a great deal of interesting and careful work which merits publication. To my regret I was unable to see that the contents of the paper justified its title. I have called "retine" the substance (or substances) present in tissue extracts which retard cell division and thus also retards malignant growth. The present paper leaves the underlying substance unidentified.

The paper strongly suggest that "promine" is a methylated amino acid, but leaves the amino acid unidentified. It supports the assumption that promotion of growth is connected with methylation, giving it an important role in the regulatory mechanism in which "retine" and "promine" are involved.

I very much hope that the two authors will find all the support which they need for the continuation of their interesting study.

A. Szent-Györgyi
Laboratory of the Institute for Muscle Research
at the Marine Biological Laboratory,
Woods Hole, Massachusetts 02543, USA

WHAT IS THE BIOLOGICAL ACTIVITY OF THE METHYLATED AMINO ACIDS?

Despite of the famous works by A. Szent-Györgyi *et al.*, "much more was at stake" about the properties of promine and retine at the present authors mentioned, and it is good to see that these studies have been continued and extended by Hungarian scientists. They have demonstrated the presence of methylated lysine and arginine in calf thymus and liver which were originally used for the source of promine and retine, and they have shown that these compounds satisfy several original criteria of promine and retine, namely in their reineckate forming ability, paper chromatographic mobility, structural similarity and so on.

This conclusion is very interesting because it is simple and unexpected. On the methodological basis their conclusion seems reasonable especially for methylated lysine, while this paper lacks the data of the biological activity of these methylated amino acids. These data might have been published in their related papers as they quoted, but I feel that detailed biological and biochemical evidences are desirable to confirm and extend their conclusion. For instance I recommend the use of tissue culture systems, if available, to obtain more quantitative and specialized results on the growth promoting and inhibitory activities of methylated amino acids. When this point had been covered we now could have a solid chemical basis of the promine-retine hypothesis.

Apart from the promine-retine theory, the present work seems important since methylated amino acids have been found in a variety of structural proteins from wide sources and nevertheless their biological roles have remained completely obscure. I expect that one of the biological functions of free and protein-bound methylated amino acids would be elucidated by the works of Drs Tyihák and Patthy.

Y. Yaoi, Ph. D.
National Cancer Center Research Institute,
Tsukiji 5-chome,
Chuo-ku, Tokyo, Japan

WHAT ARE THE PROPERTIES OF RETINE AND PROMINE?

A number of physico-chemical and chemical properties of human urine retine and promine are described. They do not match the properties one should expect from the structures reported for calf thymus retine and promine.

Introduction

There can be little doubt that the work of TYIHÁK—PATTHY (1973) constitutes a most valuable piece of analytical biochemistry. It is the purpose of this paper to analyze some of the implications of the identification of calf thymus retine and promine as methylated basic amino acids. However, a few preliminary comments appear necessary.

In the first place, retine can exist under several different forms (and the same could well be true of promine); this is to say that one can eliminate a certain part of the original "retine" molecule without destroying its growth repression properties. In this respect, it seems that Hegyeli's terminology (bound retine, [split] retine, free retine) is remarkably clear and sufficiently precise for the time being, therefore, it will be used throughout this paper.

In the second place, except as otherwise specified, all the data presented here refer to products extracted from human urine. As it will be obvious later, this point is of importance.

As the approach we personally followed was deliberately physico-chemical, so in order to be able to discuss constructively Tyihák's and Patthy's findings, we have to start this paper by a presentation of some work unpublished. It must be emphasized that this work was done in collaboration with Dr. Andrew Hegyeli.

Special techniques for the purification of retine and promine

Techniques of extraction of retine and promine from human urine have been previously described by HEGYELI *et al.* (1963a, 1963b). Two fractions are obtained, one of retine reineckate and one of promine reineckate. These fractions still contain a certain number of impurities, among which are an excess of reineckate reagent and some fatty acids; both can be eliminated for the greatest part at least, by washing with diethyl ether. Promine purity can easily be checked with the crystallographic method described in 3 because pure retine and promine reineckates belong to the cubic system while ammonium reineckate and fatty acids do not. Classical techniques of fractional crystallization do not permit one to obtain routinely on a semi-micro scale a reineckate fraction sufficiently pure for most physico-chemical studies: however, such samples can be obtained by means of a differential splitting technique and/or of a special counter-current method.

Retine can be extracted from a mixture of promine and retine reineckate by the combined use of differential splitting and floatation. All other things being equal, the rate of splitting of retine reineckate by most organic solvents is greater than the rate of splitting of promine reineckate. The specific gravity of a column of carbon tetrachloride can be adjusted with organic solvents such as chloroform or monobromobenzene so that the retine reineckate is dispersed in the bulk of the fluid, while promine reineckate remains at the top of the column. In these conditions, due to the difference in the degree of dispersion and to the difference in the intrinsic rates of splitting, retine reineckate is preferentially split and a chloroformic solution of retine can be separated by filtration. In order for the procedure to be effective, two conditions must be fulfilled. Firstly, the retine reineckate must be dry: if some crystals are wet, their rate of splitting is drastically reduced. Also, fines (especially those of promine reineckate) must be eliminated. This is easily achieved on a semi-micro scale by elutriation in a fluid such as nitrobenzene.

A preparation of higher purity can be obtained on a semi-micro or preparative scale by means of a counter-current distribution technique. Here, the first step consists of the liberation of retine and promine from their reineckate combination. This can be achieved in two ways. In the first place, the reineckate bond is split by carbon tetrachloride at room temperature; the liquid phase is concentrated under a high vacuum at relatively low temperature. Another possibility is to split the reineckates with a 1 : 1 mixture of acetone and water brought to pH 1.0 by addition of sulfuric acid. The mixture is shaken with diethyl ether which dissolves the reineckate reagent and the two phases are separated by decantation. The acetone—water phase is then distilled in order to eliminate the acetone and the water is extracted with chloroform. The chloroformic phase is cooled to —20°C in order to freeze the small amount of water which has passed into the chloroform. Finally a chloroformic solution of retine, promine and impurities is obtained by filtration.

Although highly sophisticated schemes of counter-current distribution, such as those designed by Craig *et al.* (1956), are available, they present in the case at hand the major disadvantage of providing too many fractions; in addition, some of these are very dilute. In these conditions, it appeared necessary to design a new scheme through which the number of contacts would be multiplied in order to increase the efficacy of the separation and which would produce only a small number of fractions, as little dilute as possible.

Such a scheme was patterned through a basic modification of the Jantzen extraction scheme (1932). On Fig. 1, the fundamental procedure is depicted in the case of a separation with three separatory funnels. In the Jantzen scheme, fresh solvent is added alternatively; in the new scheme, a fraction already set aside from the distribution series is recycled in place of fresh solvent. Thus a greater number of contacts is provided and a better separation with less dilution is obtained. One will note that — unlike Jantzen's scheme — this scheme cannot be represented by a two-dimensional mesh but requires a three dimensional mesh representation.

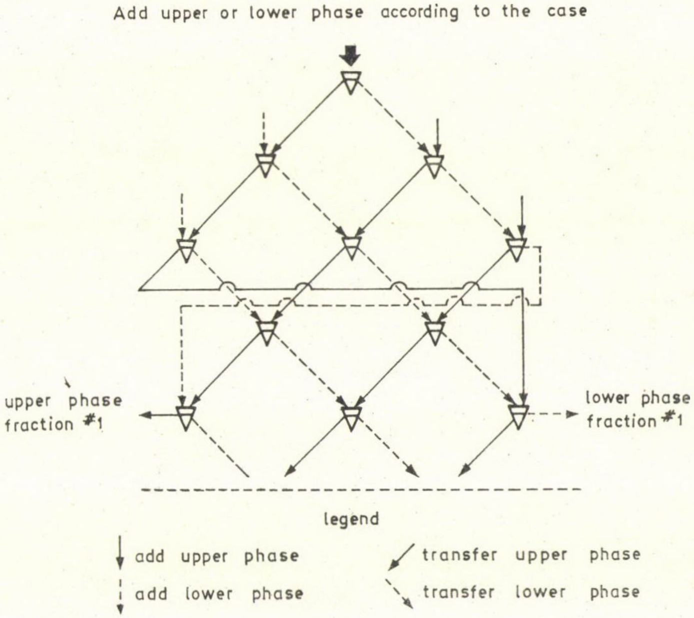


Fig. 1. Special countercurrent distribution scheme used to separate retine from promine. From Marmasse—Hegyeli

The purification of retine and promine is best carried out by distribution between a chloroformic phase and an aqueous one. At pH, the retine activity [as tested on the Krebs-2 carcinoma of Swiss Albino mice by the method of HEGYELI *et al.* (1963b)] is concentrated in the organic phase, while at pH 11, it is the promine activity which is concentrated in that phase. For routine separations, the distribution process can be monitored by UV spectrophotometry as retine and promine activities are positively correlated with the amplitude of an absorption peak around 278 nm. The first fractions obtained with this new scheme are very pure in regard to their biological activity, their Uv spectra, and the crystallographical characters of their reineckate derivatives.

Optical microscopical similarities and differences between retine and promine crystal derivatives

Retine and promine reineckates crystallize easily and by means of several successive crystallizations through which the biological activity reaches (within the limits of experimental errors) a plateau, products of sufficient purity for an optical crystallographical study are obtained. The crystals have been studied in air, in their mother liquor, in Canada balsam and in different organic solvents. Canada balsam dissolved in xylene enables one to make permanent preparations; also the xylol exerts a definite washing action and eliminates such impurities as fatty acids in appreciable proportions.

Crystals of retine and promine reineckate are very fragile. No pleochromism has been noted. Crystals prepared in the presence of formic or sulfuric acids are stable, while those prepared in an hydrochloric medium are unstable and often seem to melt at room temperature. An example is shown on Fig. 2 in which rounded edges are seen: under the microscope, inter-

Fig. 2. Retine reineckate crystals. They were prepared in an hydrochloric medium and mounted in Canada balsam. The photography was taken with parallel oblique illumination (artificial daylight)

ference fringes around such crystals are often noted. The two aspects of retine reineckate which are most frequently encountered are shown on Figs 3 and 4 and will be referred to here as the ruby shape and the cube shape. These two shapes are isomorphic, as can be deduced from different orthogonal projections (Figs 4 and 5). Such crystals can be visualized as cubes truncated by planes parallel to the edges. Retine and promine reineckates belong to the class m3m [m 3 m in BUERGER's notation (1956)], a typical group of such crystals in the ruby morphology is shown on Fig. 2. A model of promine reineckate in the cube morphology is shown on Fig. 6.

Retine reineckate crystals can be differentiated from promine reineckate crystals by

1. their colour: promine reineckate is a deep red, retine reineckate is pale (when the crystals are smeared on a microscope slide) and sometimes appear pink or greenish;
2. their size: promine reineckate crystals are consistently larger than retine reineckate crystals.

In certain preparations, and especially at the onset of crystallization, retine and promine crystals appear in tabular form. In that case they can be distinguished (with some experience) by their colour and their size. Penetration twins of retine reineckate crystals are often found, e.g. two cubes twinned on 111. We have also encountered many cases of twinning along 100.

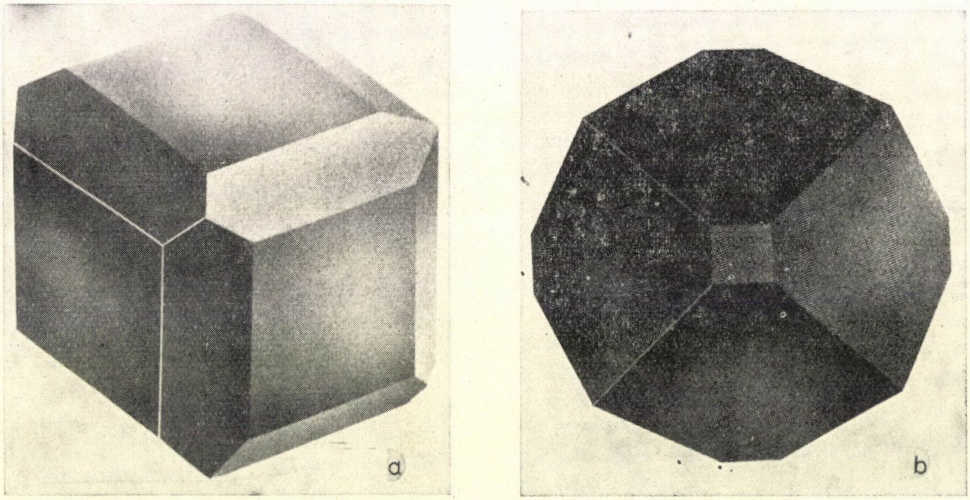


Fig. 3. Two aspects of retine reineckate crystals: *a)* cube shape; *b)* ruby shape

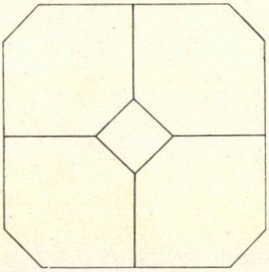


Fig. 4. Orthogonal projection of retine reineckate in the ruby shape on a plane parallel to a square facet

In the latter case, the crystals were generally unequal in size, which makes the phenomenon difficult to observe.

Although retine and promine reineckates belong to the cubic system, many preparations contain impurities such as fatty acids and in that case some birefringence is noted. The birefringent impurities can often be eliminated (except when they are locked within the crystals) by careful washing with organic solvents such as ethyl ether or 2-butanol. The property of isotropy of retine and promine reineckate crystals provides an easy and sensitive test for the purity of the samples and has been used routinely as a control of analytical separation. When a preparation of retine reineckate is contaminated with anisotropic substances, or when the impure (?) crystals of retine reineckate being to split, positions of extinction are generally observed as indicated on Fig. 8.

The refractive index of promine and retine reineckate derivatives has been determined by the immersion method, with axial and oblique illumination, and taking advantage of the Becke effect. Artificial daylight produced by an interference filter was used. It was found that the refractive index of retine and promine reineckate lies within the range 1.55 (monobromonophthalene) to 1.74 (methylene iodide).

A few other crystal derivatives of retine and promine were also studied. The chloroplatinates of retine and promine are indistinguishable by optical crystallography; they both belong to the point group m3m. Unfortunately this property does not seem to be very useful for the control of the purity of a preparation (as it is the case with the reineckates) because some impurities crystallize also in the same system. A preliminary study of the chloroaurate derivatives did not reveal any striking difference between the promine and retine compounds. In addition, the chloroaurate samples are rather unstable.

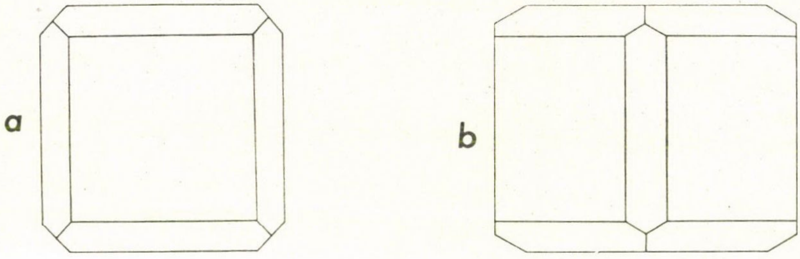


Fig. 5. Orthogonal projections of retine reineckate in the cube shape: *a)* projection on a plane parallel to a square facet; *b)* projection on a diagonal plane

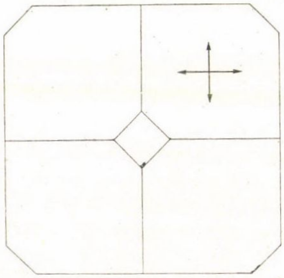


Fig. 6. Positions of extinction often found with retine reineckate crystals contaminated by some anisotropic material or undergoing a chemical splitting

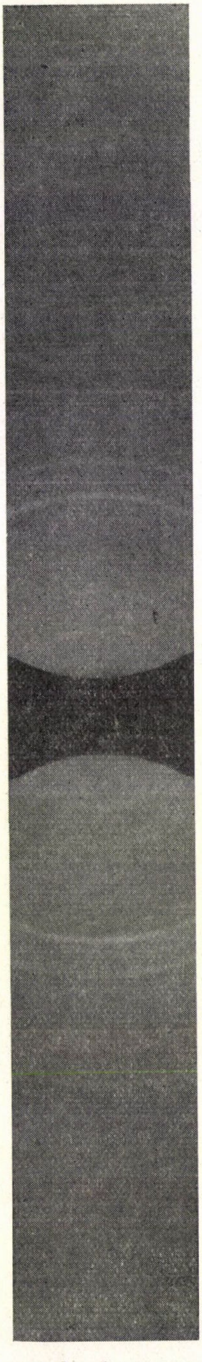
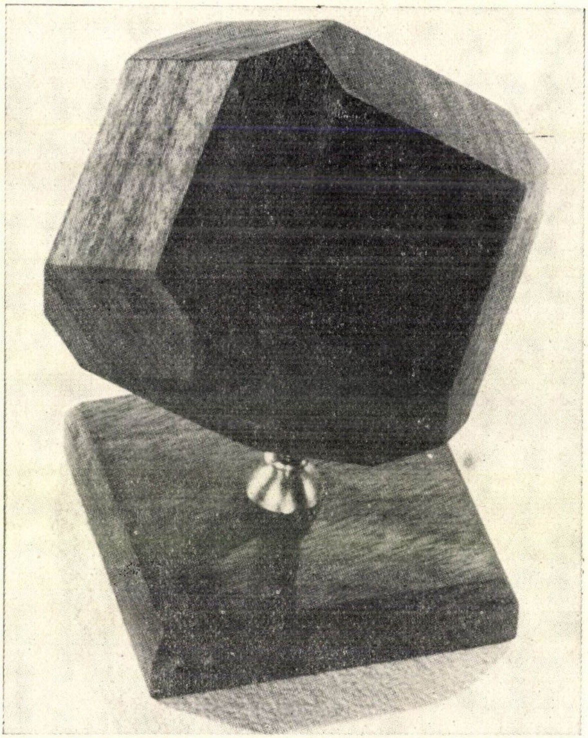


Fig. 7. **X-ray powder diagram of a sample of retine reineckate of a very high purity: Cu(K) radiation, 45 kV, 15 mA, exposure time 10 hours, cylindrical camera (Diameter: 143.2 mm). 19 lines were visible on the film. Picture taken by A. O. KING**



Fig, 8. Model of a single crystal of promine reineckate

The molecular weight of retine. Number of reineckate residues per retine molecule

As retine reineckate belongs to the cubic system, it could be expected to give a not too complex X-ray diffraction spectrum from which an estimation of the molecular weight might be drawn.

Retine and promine were extracted from a few thousand liters of human urine, kindly furnished by the Otic U.S. Air Force Base. They were concentrated and a small aliquot chromatographed on paper. One of the spots eluted had a very high retine activity. Its reineckate derivative was crystallographically homogeneous as far as isotropy, crystal shape and colour are concerned. X-ray diffraction spectra were obtained using Cu(Kα) radiation (45 k^{v}, 15 mA). Due to the great absorbing power of the material, exposure times of the order of 10—15 hours were necessary; the type of pattern obtained is shown on Fig. 7. It can be used to obtain an estimation of the molecular weight of retine, as follows; if it is assumed — as is often the case — that the first line (smallest angle) correspond to reflections from the planes 100, 010 and 001, the length of the side of the unit cell is equal to the interplanar spacing distance, i.e. 9,400 A. Therefore the volume of the unit cell is 831×10^{-24} cm^3.

On the other hand, the specific gravity of retine reineckate is lower than 1.595 but higher than 1.500, as determined by the floatation method. Taking as a first approximation the median of these values, that is to say 1.55, the molecular weight of retine reineckate is given by

Table 1

Estimates of the molecular weight of retine computed with a unit cell of 9.40 A and a specific of 1.55

Number of retine reineckate molecules per unit cell Number of reineckate residues bound to one retine molecule	1	2	≥ 3
1	458	70	<0
2	139	<0	<0
≥ 3	<0	<0	<0

Table 2

Upper estimates of the molecular weight of retine computed from X-ray diffraction data

Number of retine reineckate molecules per unit cell Number of reineckate residues bound to one retine molecule	1	2	≥ 3
1	480	81	<0
2	161	<0	<0
≥ 3	<0	<0	<0

$$M = \frac{1.55 \times 831 \times 10^{-24}}{1.66 \times 10^{-24} \times n}$$

where n is the number of molecules per unit cell. Therefore

$$M = 775/n\,.$$

The molecular weight* of the reineckate residue is 318.4. On Table 1 are listed the various possible combinations for the number of retine reineckate molecules per unit cell and the number of reineckate residues bound to one retine molecule. Simple arithmetic shows that the two lower positive values listed in Table 1 are impossible. In these conditions the only possibility left is that of one monoreineckate per unit cell.

One reaches the same conclusion if one examines the estimates of the molecular weight of retine listed in Table 2. These estimates represent upper limits because:

a) the indexing selected is the one which gives the largest possible unit cell for the X-ray diagrams obtained;

b) they were calculated with an upper limit for the specific gravity.

These data suggest that the molecular weight of retine is ca. 450, but certainly not higher than 480. This is in agreement with data previously reported by HEGYELI *et al.* (1963b): the

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* Computed with the International Atomic Weights of 1959.

elution patterns of a gel filtration on Sephadex of a retine preparation suggested a molecular weight of around 400.

One will note that the existence of only a mono-reineckate of retine is established. The implications of this most important fact are discussed in §. 6 of this paper.

Some properties of promine

a) *Preparation of promine reineckate monocrystals*

Fresh human urine was extracted with an equal volume of chloroform: the organic phase was concentrated to 1 : 15 of its original volume in a film evaporator under reduced pressure and refrigerated overnight. The precipitate which appeared was collected on a Buchner funnel, washed with cold distilled water and extracted by refluxing with dilute acetic acid (glacial acetic acid 20%, distilled water 80%). The mixture was cooled to room temperature and the insoluble material eliminated by filtration. The clear filtrate was distilled under reduced pressure (0.1—0.2 Torr) in a flash evaporator. Two volumes of a saturated (at room temperature) aqueous solution of ammonium reineckate were added to the distillate; the solution was then concentrated to 1 : 10 or its volume. The crystallization started during the concentration process and was completed in 1—2 days at 4°C. The crystals were collected on a Buchner filter and washed with cold water in order to eliminate the unreacted reagent. The fatty acids were eliminated by washing with ethyl ether.

The crystals were treated with 50% acetone in order to split the reineckate derivative. The solution was filtered and the filtrate treated in a separatory funnel with an equal volume of a 1 : 1 mixture of distilled water and ethyl ether; 50% sulfuric acid was added to the mixture until all the reagent passed into the organic phase. The aqueous phase was separated, washed with ether and concentrated *in vacuo* to a small volume. It was then extracted several times with an equal volume of chloroform. The chloroformic solution was extracted with an alkaline aqueous solution (pH 9—10), the water phase was cooled to 2—4°C and the pH brought to pH 1—2 by addition of formic acid. Two volumes of a saturated (at room temperature) solution of ammonium reineckate were added and the mixture concentrated until the first crystal appeared. The mixture was then warmed up until complete dissolution of the crystals and the crystallization was allowed to proceed.

The crystals were collected on a fritted glass filter, dried at room temperature and dissolved in distilled water. Fractional crystallization was performed using standard techniques. The last crystallization was carried out at room temperature, in an environment relatively free of vibrations, and proceeded for more than 6 weeks. The best crystals were mechanically removed.

At all stages of the purification, the reineckate crystals obtained were examined by the techniques of optical crystallography described above. The absence of retine reineckate contamination, which we have found to be frequent, was checked by a differential splitting technique: retine reineckate is split by a short treatment with carbon tetrachloride, while promine reineckate is not.

The bioassay of promine was carried out as previously described for retine by HEGYELI *et al.* (1963b). The reineckate derivative was split with 50% acetone and tested on the Krebs-2 carcinoma of the Swiss albino mice: the tumour promotion activity was the strongest ever observed with such a preparation. Fig. 8 shows a model of a mono-crystal of promine reineckate.

b) *X-ray diffraction analysis*

A rather complete analysis was performed by KING but we shall quote only a few results relevant here. In the first place the space group was found to be cubic (space-group Pn3m —O_h^4) in accordance with the observations of optical crystallography reported in 3. The unit cell was determined as shown on Table 3 and Fig. 9.

Table 3

*Analysis of the X-ray powder diagram of promine reineckate. Cu(Kα) radiation, Ni filter, camera diameter: 143.2 mm * denotes reflections characteristic of a primitive lattice type. The analysis was carried out on the basis of a value a = 13.166 A for the lattice constant, as determined from an analysis of the zero-level Weissenberg diagram*
(From A. O. King)

Intensity	d_{obs} (Å)	(hkl)	d_{calc} (Å)
s	9.321	110	9.311
w	7.381	111*, **	7.602
m	6.601	200	6.583
w	5.336	211	5.376
m	4.652	220	4.656
vvw	4.235	510	4.164
vs	3.792	222	3.801
vw	3.663	320*	3.652
w	3.520	321	3.519
ms	3.290	400	3.291
mw	3.106	330	3.104
w	3.021	331*	3.020
vvw	2.895	421*	2.873
vvw	2.791	332	2.807
vvw	2.712	422	2.687
w	2.599	431, 510	2.582
vw	2.471	432*	2.445
vw	2.400	521	2.404
w	2.328	440	2.327
vvw	2.260	334, 530	2.258
vvw	2.206	600	2.195
vvw	2.046	541	2.031
w	1.988	622	1.985
w	1.904	444	1.900
vw	1.858	543, 550	1.862
vvw	1.791	552, 633, 721	1.792
vvw	1.741	544, 722*	1.744
vvw	1.691	643*	1.686
vvw	1.658	800	1.646
vvw	1.583	742*, 821	1.585
w	1.551	660	1.552
vvw	1.531	743, 750, 831	1.531
w	1.511	662	1.510
vvw	1.476	840	1.472

** See Fig. 9.

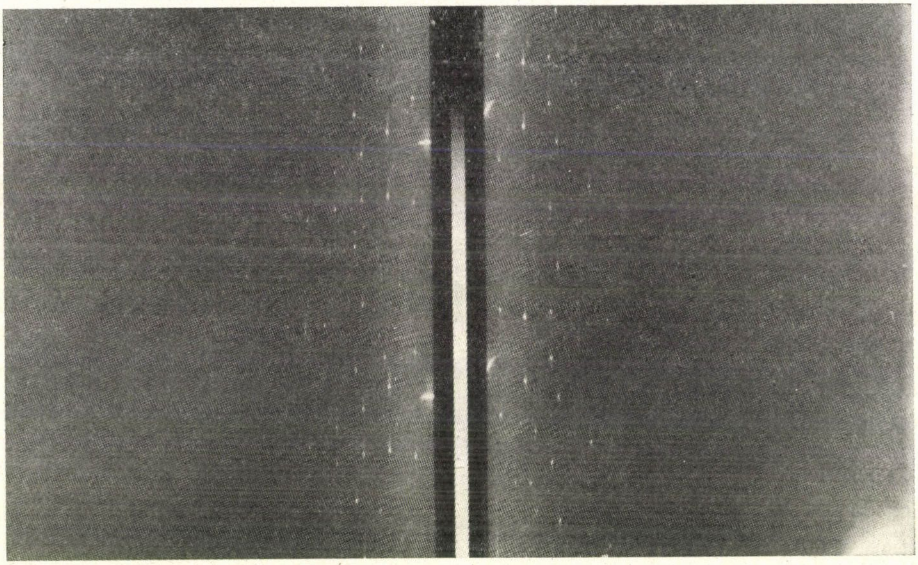

Fig. 9. 1st-level equi-inclination Weissenberg diagram of promine reineckate. Note the very strong reflection (111) near the center line; from its presence it can be inferred that the crystal lattice belongs to a primitive type. Cu(K) radiation, Ni filter, camera diameter; 57.3 mm, $\mu=3.5°$, exposure time: 5 days. Courtesy of A. O. KING

One will note that it is much larger than the unit cell of (human urine) retine reineckate. This is a very important property as it is one of the very few physico-chemical differences observable between the reineckate derivatives of retine and promine.

Discussion

Let us note in the first place that we do not possess at the present time proof of the unicity of retine and promine, that is to say we do not know with absolute certitude if it is, for instance, the same tumour-growth inhibitor which is present in mushrooms, in clams and in human urine, just to quote a few known sources of biologically active compounds. All we can state, in truth, is that they likely belong to the same chemical family as they behave in the same way with respect to some group reagents: for instance they form reineckate and chloroplatinate derivatives. The concept of the unicity of retine and promine is certainly biologically attractive, but until its correctness is definitely established, we must not attribute to it more credit than to any other scientific hypothesis. In other words, we should be careful not to create another biological dogma. Clearly, in order to analyze and to prove or to disprove this possibility, one will have to turn systematically to the determination of some physico-chemical parameters such as those described here.

As a matter of fact, the author is very doubtful that N^G-N^G-dimethyl-arginine and ε-N(trimethyl)-lysine respectively identified (TYIHÁK—PATTHY 1973) as calf thymus retine and promine for the following reasons:

1. As we have seen in 4, the analysis of X-ray data enables one to show that retine can form only a mono-reineckate. This is certainly consistent with a mono-amino derivative; however, IR spectra (MARMASSE — HEGYELI), show an imonium bond.

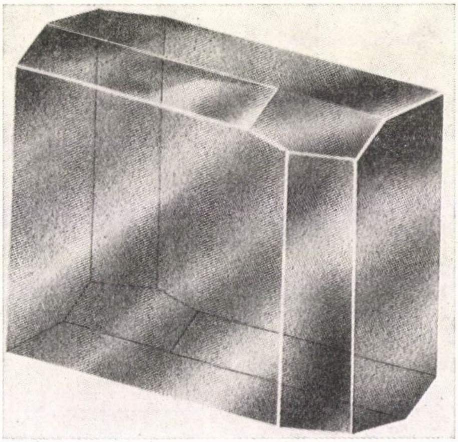

Fig. 10. Artist view of the 2,4-dinitrophenylhydrazone of free retine (carbon suboxide)

2. The molecular weight of $N^G-{}^GN$-dimethyl-arginine is much smaller than the molecula weight of human urine retine (see §. 4 of this paper).
3. The IR spectrum of a very pure solution of human urine retine in chloroform is very close to that of cis-5-cholestene-ol 3-one 11 (MARMASSE — HEGYELI). It also shows an intense and narrow band in the far infra-red (ca. 533 cm^{-1}) which is typical of a sterol structure. The UV spectrum in organic solvents such as chloroform or hexane suggests a structure of homoannylar dience, while the absence of a B band enables one to eliminate the possibility of a pyridine substituent in C_{17}.

It can be noted too that human urine retine and promine give at least some of the colour reactions of the sterols. One of these reactions, the Salkowski reaction, enables one to distinguish between retine and promine on chromatograms: only the promine gives a strong positive reaction with a 40% ammonium pentachloride solution in chloroform.

In these conditions, human urine promine and retine should be respectively a hydroxy-ceto-sterol and a dihydroxy-sterol.

4. Would the two methylated amino acids show the pH dependence solubility described in §. 2?
5. Would a carbonyl band* appear in the IR spectrum of N^G-N^G-dimethylarginine, and by acetolysis would a compound (carbon suboxide) be released, whose 2,4-dinitrophenylderivative possesses the very special shape shown in Fig. 10?

We are confronted with many (apparently) contradictory data, which, however, fall in well-defined groups; it seems then, that the best way out of this very embarrassing situation is, at least tentatively, to delete the concept of the unicity of promine and retine, which possibly does not have more than a dubious metaphysical value.

It may well be that an oligopeptide moiety is present in human urine retine and promine. But in that case the amino groups should be blocked (cyclic oligopeptidic moiety?) and in addition an imonium bond must be present. From this point of view, one will note that the acidic hydrolysis commonly used to liberate retine is likely to induce a serious loss of tryptophane for instance. Clearly, some more work needs to be done along this line.

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* The existence of a carbonyl band was known long before EGYÜD's paper (1965). See SZENT-GYÖRGYI (1964).

In this respect, there are a few other points which should be touched upon, and boren in mind. At present, retine and promine have an operational definition; no more. Many compounds can be expected to show some of these properties. For instance, a few years ago, KWON—OLCOTT published a very interesting paper on malonaldehyde (1966); but in spite of the similarities between this compound and (human urine) free retine, the latter just cannot be identical as pointed out by OLCOTT—KWON themselves. On the other hand one should not forget the fantastic interplays and possible interconversions of metabolites. In other words, we should be prepared and looking for new developments in the dynamic aspects of tumour metabolism.

Acknowledgements

I would like to acknowledge the courtesy of Dr. Tyihák and Dr. Patthy for the advanced communication of their paper, as well as the kindness of Dr. Pál, managing editor of Acta Agronomica, who invited me to write this review. As mentioned at the beginning of this paper, I have used here many results obtained in collaboration with my old friend Dr. Hegyeli, and which are still largely unpublished. I would like to thank also for their collaboration Mr. King (X-ray diffraction spectra), Mr. Sousa (IR spectra), and Mr. Bailey (artist views of crystals).

C. MARMASSE
Biophysical Laboratory, Faculty of Sciences,
National University of Mexico,
U.N.A.M. Mexico 20, D.F.

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IS THE INHIBITING EFFECT OF METHYLIZED ARGININES ON CELL PROLIFERATION A PROVED FACT?

The two authors of the paper aimed at no less than clarifying the chemical structure of the growth stimulators and inhibitors discovered by Szent-Györgyi and his collaborators. The task is all the more complicated as the authors possess no original promine and retine as a basis for comparison. So they are under the necessity of producing fractions possibly corresponding to promine and retine from various biological objects by the methods described in the literature.

The clarification of the physico-chemical properties of the fractions thus obtained finally led them to the conclusion of considering the methylized lysines identical with promine and the methylized arginines with retine. The speculation seems logical, but it can be accepted as a fact only when all the biological activities of promine and retine are proved to exist in the above substances too. According to our own biological investigations the methylized lysines do stimulate the proliferation of tumour cell populations and that in a way as forcing a part of the so-called resting cells to reenter the cell cycle. Considerably less data are, however, available on the biological action of methylized arginines. The authors' statement relative to retine can only be accepted when the inhibiting effect of methylized arginines on cell proliferation is proved true. Further on, it would be necessary to demonstrate by the joint application of the two groups of compounds that these substances are really antagonistic to one another. Examinations of this nature require large quantities of matters, but the importance of the question would justify the production of a larger amount of pure methylized arginine.

It should be noted further, that besides the known inhibitors of cell proliferation there are a number of compounds and tissue extracts which stimulate the proliferation of certain cell systems.

In our opinion, the promine-retine-like effect cannot be restricted to a single group of compounds, and the action of the already known stimulating and inhibiting compounds can be generalized only to such an extent as made possible by experiments performed with various test objects.

B. Szende
Semmelweis Medical University,
I. Institute of Pathology
1085 Budapest, Üllői út 26.

ARE THE N-METHYL-GUANIDO-ARGININES IDENTICAL WITH THE GROWTH RETARDING COMPOUNDS?

I read the article of E. Tyihák and A. Patthy entitled: On the chemical nature of "promine" and "retine" with great interest. The authors certainly made a carefully detailed study on growth promoting substances, ε-N-methyl-lysines (called "promine"), further supporting the previous observations reported in Nature during 1970 and 1971.

To my great regret I am unable to see that the contents of this paper fully justify its title in reference to the retarding substance(s) of cell division (called "retine"). The authors claim that N-methyl-guanido-arginines are identical with the growth retarding compounds. Their conclusions are apparently derived from highly speculative approaches and the authors omit to present biological datas in support of their findings. Instead, they quote references which are not readily available (i.e.; TYIHÁK 1964a, 1972, TYIHÁK—SZENDE 1969—1970) and further state on page 17 that: "The retine character of guanidino-methylated arginine derivatives will be unambiguously decided by current biological investigation". Thus the report leaves the underlying retarding substance(s) unidentified.

The article is a summary of research on widely claimed growth retarding and promoting substances present in a variety of tissues without the proof of their chemical identity, supplemented with original work, which merits publication, on chromatographic separation, identification and distribution of a number of methylated basic amino acids in beef liver and thymus.

The report also lends a strong support, although leaving the compounds involved in ambiguity, to recent observations that cell division is connected to methylation processes.

I sincerely hope, that the authors will clarify questions remained unanswered of their research endeavour.

L. G. EGYÜD
Institute for Muscle Research,
P.O. Box 342
Woods Hole, Mass. 02543, USA

DO THE METHYLATED DERIVATIVES OF BASIC AMINO ACIDS EXIST IN A FREE STATE IN VIVO, OR ARE THEY ARTEFACTS?

The research work done by the authors is highly valuable as they offer a deeper insight into the investigations on calf thymus and liver, carried out by Szent-Györgyi and co-workers. I agree with the applied methods and techniques and consider them adequate. As for the material used in the experiment and the conclusions drawn, I make some reservations. I do not see it convincingly proved that the examined amino acid derivatives exist in a free state under in vivo conditions.

Investigations in this field up till now unanimously reveal that the so-called post-synthetic biological modification of certain proteins may perform a sgnificant regulatory function. In each case phosphorylation, glycosidation, hydroxylation or thiolation are achieved post-synthetically, that is to say, modification occurs on the completed or proceeding protein polymer. The authors demonstrated the derivatives of the identified amino acids in free form. There is a possibility that the presence of derivatives of the two amino acids in the organs studied — calf thymus and liver — is a result of a high protease of proteolytic activity. In other words, these amino acids are produced artificially, in the first place by the proteolytic decomposition of histones. May I remind the authors of the considerable protease activity they have shown in thymus nuclei, decomposing the nucleohistones, first of all the histone fractions f1 and f3. Other histone fractions are decomposed only when they are dissociated from DNA. The methylated basic amino acid is presumably released from fraction f3 and, may I add, that storing of thymus — even when in a frozen state — enhances the release of the amino acids examined.

It would be very important to know the time elapsed from obtaining the organs from the slaughter-house to completing the preparations, on one hand, and the conditions and speed of cooling of the excised organs, on the other. The stop of blood supply, pronounced anoxaemia may, namely, provoke protease activity, and the speed of cooling is in close correlation with the location and extent of the provocation.

While appreciating the valuable new results reported in the present paper, I consider the additional investigations suggested to be necessary and important in order to elucidate, whether the amino acid derivatives examined are, in fact, existing in a free state of they are artefacts.

F. Antoni
Institute of Medical Chemistry,
Semmelweis University of Medicine,
1088 Budapest, Puskin u. 9.

CAN ALL THE METHYLATED ARGININE BE ATTRIBUTED TO THE THYMUS HISTONE FRACTION?

Throughout the paper by Tyihák and Patthy the distinction between the methylated free amino acids and the methylated protein has not been made. One is never clear what is meant by sentences similar to that of the last paragraph of the Introduction viz. ". . . further on a methodological basis and on grounds of partial biological investigation results already published try to prove that methylated basic protein-amino acids are identical with promine and retine".

It is my opinion that Tyihák and Patthy should not claim identity between the isolated methylated amino acids and the substances "promine and retine". The latter are hard to characterize because they are not homogeneous preparations as obtained from calf thymus and liver. Perhaps samples of "retine and promine" as isolated by the Woods Hole group from urine could be obtained from New England and then characterized by Tyihák and Patthy. As of now, the authors can only give an opinion on the strong similarity of retine and promine with methylated basic amino acids — nothing more.

Perhaps the strongest point of this paper is that dimethyl-arginine in the thymus of a healthy calf is found in substantial quantity. Tyihák and Patthy should be able to calculate whether all the methylated arginine could be attributable to the thymus *histone* fraction. This calculation is possible from the substantial data on the subject published by E. Smith's group. It is likely that all of the histones may not be able to account for the total quantity of methylated arginine present in the thymus. This would strengthen their claim concerning effects of methylated amino acids on cellular growth.

As for trimethyllysine (TML) — are the authors aware that this substance is converted to carnitine by various cell types and the putative effects of TML on cell growth could arise from deficiencies of carnitine in the growth medium affecting cell membranes? (JBD 248, 2170; 2176, 1973).

The authors have shown that methylated amino acids are present in plant and animal cells. They have not been able to clarify whether these amino acids are normally found free within the plant and animal cells. If they are not free then are they associated with proteins other than the histones? Are these amino acids found in the cytosol as a result of turnover of proteins such as the ribosomal structural proteins? Alternatively, are these amino acids associated with some glycosylated proteins which are secreted by cells for any of diverse functions like hormonal, structural, enzymatic, transport, lubrication or cellular adhesion? Or are they incorporated in lipids via carnitine metabolism?

The authors have made a case for showing similarities of promine and retine with those of methylated amino acid derivatives. The authors have not resolved the chemical nature of promine and retine because promine and retine have not been shown to be homogeneous preparations.

M. Reporter
Charles F. Kettering Research Laboratory,
150 East South College Street,
Yellow Springs, Ohio 45387, USA

IS THERE ANY RELATIONSHIP BETWEEN THE ACTION OF PROMINE AND RETINE AND THE SHAPE OF THE PLANT?

Looking at the question from the side of plant organization we must consider that ε-N-methylated lysin (promine) and methylated arginin (retine) are found in the root of red beet (*Beta vulgaris* var. conditiva) which differs from the general form of root organization in developing a strongly thickened root-top. Namely, collateral vascular bundles are formed here in several circles through the outward development of successive cambial rings in the parenchyma of the cortex. This means that the parenchyma cells have a high potential capacity for cell division which may be in connection with the presence of the above mentioned substances.

Considering that the authors performed the examination of red beet in all probability at the fully developed stage of the root-top the question arises, what the quantitative composition of the examined substances is like in the different phases of development, starting with the not yet thickened rootlet of the seedling. Namely, this would give some answer to the question to what extent these substances influence the thickening of the root of red beet. What is the relationship e.g. between cambium formation and the stabilization of transporting tissues on one hand, and the quantitative conditions of the above substances, on the other, considering that cell division and cell stabilization (cell elongation) alternate each other phase-like?

Another problem is represented by the fact that only the upper part of the root of red beet thickens, where these substances are supposed to be present in larger quantities and in some state of equilibrium, though probably not in equal quantities: the amount of ε-N-methylated lysin and methylated arginin found at a fully developed stage should be compared to the quantitative conditions existing in different development phases in the upper thickened part of the root. It is probable that these substances causing cell proliferation, tumours, are not present in large quantities in highly meristemic tissue regions: in the growing tip of the root and its immediate neighbourhood, while larger and efficient quantities are found of them in cells in the process of stabilization (in the upper, elongating zone of the root).

It can be supposed that different quantitative ratios may exist in the individual phases of development between the two substances. A definitely larger amount of ε-N-methylated lysin causes an extent of cell division when the produced cells still pass over to a state of differentiating transporting tissue cell. With a quantity larger than that — while the intensity of cell division may be maintained or even increased — the elongation, stabilization and differentiation toward a certain activity of the cells after cell division (transportation) will no longer follow. Similar results were obtained in our experiments when under the influence of growth regulators (gibberellin precursors: 2 CH-9 fluorenole-9-carbonic acid-methylesther, or 9 OH-9 fluorenole-9-carbonic acid-butylesther) the main root of bean increased some 100 per cent in diameter during germination, but no laterals developed, and compared to the one cell-row pericambrium in the root of the control plants — which is responsible for the initiation of laterals — a 18—20 cell-row meristemic undifferentiated homogeneous tissue zone developed.

It would be interesting to examine the ε-N-methylated lysin and methylated arginin contents in roots of other species (*Petroselinum, Daucus, Brassica oleracea* L. var. *gongyloides* L.) parallel with a study on non-thickening roots of related species, to find out whether the thickening of roots is accompanied by a larger amount of these substances.

It has been raised that the quantitative conditions of ε-N-methylated lysin and methylated arginin found in liver would be worth being examined in livers showing tumours, cancerous disorders. It should be found out how much the quantitative ratio between the two substances shifts in this case. Furthermore, a study of this kind would give a picture of how far these substances are responsible for keeping the functioning of this organ in balance, and to what extent the *in vivo* tissue proliferation and tumour formation depend on their quantitative conditions.

P. Gracza
University of Horticulture
Department of Botany and Botanical Garden,
Budapest

WHAT IS THE CHEMICAL NATURE OF PROMINE AND RETINE?

This reviewer is an organic chemist who has only temporarily been involved in his former teacher's, Albert Szent-Györgyi's, research on the chemistry of retine. In a paper (Fodor—Sachetto—Szent-Györgyi—Együd 1967) quoted by Tyihák—Patthy (1973), we described our proof by spectral and chemical methods, that the α-keto aldehyde, isolated (Együd—McLaughlin—Szent-Györgyi 1967) from liver in the form of its 2,4-dinitrophenyl hydrazone, was 3-deoxyglucosulose (*1*). An improved synthesis of *1* has been elaborated and the product and its derivatives were unequivocally identified with *1*.

Since 3-deoxyglucosulose showed no tumour-retarding activity, *1* could not be retine. Furthermore Szent-Györgyi (1967) assumed that *1* was an artefact. Kato *et al.* (1970) showed more recently that 3-deoxyglucosulose is present in calf liver of the living animal and is accumulated after death, thus excluding the possibility of it being an artefact. The biological significance of *1* is, nevertheless, not related to tumour inhibition. Therefore, Szent-Györgyi and I lost interest in that substance.

Tyihák and Patthy have now succeeded in isolating a number of N^{ε}-methyl derivatives of α,ε-diaminocapronic acid and of α-amino-δ-guanidovaleric acid from calf liver and thymus. In paper chromatography symmetrical δ-N,N-dimethyl L-arginine (*2*) showed R_f values within the range indicated for retine (Szent-Györgyi *et al.* 1962). At the same time, the N^{ε}-trimethyl-L-lysinium sal (*3*) did not move from the origin, similar to the behaviour of promine. Furthermore, formation of a precipitate with Reinecke's salt from the respective N-methylated basic amino acids occurred under similar circumstances as it did with retine and promine.

More significantly, Szende *et al.* (1970), found tumour growth promoting effect of *3* (and of the partially N^{ε}-methylated lysins). In addition, in the summary of the recent paper they state that L-arginine, methylated in the guanido function *has** a tumour retarding activity. This strong statement is at variance with the prospect mentioned later in the paper, namely that the "retine character of guanidino-methylated arginine derivatives *will be* unambiguously decided by the current biological investigations".

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* Italics by the reviewer.

This reviewer thinks that the isolation from liver and the discovery of promine and retine-like activity of certain N-methylated basic amino acids are very interesting. These substances may even become relevant in solving fundamental questions of growth promoting and retarding bioprocesses. However, it is very difficult to accept the statement as to the *identity* of promine with N^{ε}-methyllysines and of retine with δ-N-methylarginines. Identification has to be based on comparison of two (or more) chemically defined compounds by unequivocal methods as the authors themselves recognize it on another page of their paper. However, similar R_f values, the fact of precipitate formation with Reinecke's salt, and comparable biological activities do not satisfy either of those criteria, so there is not enough evidence for identifying retine with the methylarginines. This is even more true for promine and the N^{ε}-trimethyl lysine salt, which were compared, *inter alia*, based on the observation that none of them moves from the starting line in paper chromatography. Most quaternary betaines and their salts do not move from the origin. Therefore, this fact is neither a specific characteristic of promine nor of the authors' quaternary ammonium salts.

The only statement this reviewer would make is that the similarities (*a*) in the method of isolation, (*b*) of some physical constants, and (*c*) in comparable biological activities can be regarded as *circumstantial* evidence for the probable chemical nature of promine and of retine.

G. Fodor
Department of Chemistry, West Virginia University
Morgantown, West Virginia 26506, USA

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IS IT POSSIBLE THAT THE "RETINE" ISOLATE SHOULD CONTAIN METHYLATED AMINO ACIDS IN SUCH AN AMOUNT AS SHOWING A BIOLOGICAL EFFECT WITHOUT BEING DEMONSTRABLE CHEMICALLY?

Since Szent-Györgyi raised the idea of substances influencing cell division being present in every cell (Szent-Györgyi 1960, Szent-Györgyi—Hegyeli 1962, Szent-Györgyi—Hegyeli—McLaughlin 1962) important scientific work has been carried on in this field. In spite of this, any attempt made to prove the existence of substances controlling cell division — called by Szent-Györgyi "promine" and "retine" — has so far failed. As in the paper written by Tyihák E. and Patthy A. the attempts made to isolate "promine" and "retine" and expose their structure are discussed in detail, on the request of the editorial office we only wish to make some remarks on the subject.

In spite of the fact that for the time being "promine" and "retine" could be most unambiguously characterized by the process of isolation itself, not even two of the methods found in the literature for the extraction of the mentioned substances are identical. In theory "promine" and "retine" could be unambiguously characterized by their biological action as well, however, the preparations so far used in the biological experiments always contained both components and that at an undefined ratio, so the results could not — even in Szent-Györgyi's opinion — be evaluated (SZENT-GYÖRGYI—EGYÜD—MCLAUGHLIN 1967). Therefore Szent-Györgyi and Együd and their collaborators, respectively, later endeavoured to isolate "promine" and "retine" in a pure state then determine their structure by physical and chemical methods. The common aim in the numerous isolation methods used by them was to remove the water soluble components — e.g. amino acids — from the tissue extracts to a possibly maximum extent. From the highly acidic solution "promine" and "retine" were usually extracted by chloroform, and detaching with Reinecke salt or other steps of isolation were carried out from the chloroform solution. On the other hand, Tyihák and Patthy used extracts obtained with physiological salt to separate in the form of Reinecke salt the substances later subjected to isolation by chromatography.

Együd and his collaborators (FODOR—SACHETTO—SZENT-GYÖRGYI—EGYÜD 1967) endeavoured to identify "retine" as a glioxale derivative. We too made similar investigations (KARÁDY—PRÓKAI—HALMOS 1971) with substances of antihistamine action found in the cells (resistin), and through the kind assistance of Együd and his collaborators had an opportunity to compare the substances isolated by us with those synthetized by them. The obtained glucosulose was not identical with the substance isolated by us either in action or in chemical structure.

In the course of our investigations we have arrived at the conclusion that our substance originates from the sugar components of cells undergoing a transformation in the highly acidic medium (HCL) (Lobry de Bruyn, van Ekenstein reactions). In 1967 Szent-Györgyi too ascertained (SZENT-GYÖRGYI—EGYÜD—MCLAUGHLIN 1967) that the substance isolated by Együd and his collaborators and identified as glucosulose was again a synthetic product (FODOR—SACHETTO—SZENT-GYÖRGYI—EGYÜD 1967).

In spite of the fact that Szent-Györgyi, Együd and their collaborators could not isolate retine in a pure state and did not sufficiently prove its structure either, the possibility of the isolate concerned containing glioxale derivatives of "retine" action too cannot be excluded. At the same time, it seems improbable that the methylated amino acids suggested by Tyihák and his collaborators are present in this isolate in such large quantities as showing a biological effect without being chemically demonstrable.

If we start from the fact that numerous groups of compounds show a cytostatic effect it seems reasonable to look for "retine" among the natural representatives of these compounds, first of all among the methylating substances studied in some detail. An important endeavour of recent years is to clear up the correlations between the methylated derivatives found in the living organism.

In this context the methylizing enzymes and enzyme systems recently found in the cell nuclei appear to be of basic importance. Paik and Kim (PAIK—KIM 1968) pointed out in calf thymus the protein methylases which by the aid of S-adenosyl-methionine methylate the free amino groups in the basic amino acids of histones. Methylated arginine and lysine produced in the course of the *in vivo* decomposition of histones have been pointed out in human urine as well (KAKIMOTO—AKAZAWA 1970). Unfortunately, on the basis of the description given by Tyihák and Patthy it cannot be decided for certain whether the basic amino acids pointed out by them may really be contained in a free state in the cells, or were produced in the course of isolation by the enzymic decomposition of histones. According to our present knowledge the Stedman—Stedman hypothesis (STEDMAN—STEDMAN 1950) seems to be valid, namely, it is the histones rather than low molecular weight substances that play a decisive role in controlling the protein synthesis and thereby the cell division. (On the basis of methylated amino acids being excreted with urine, and with the known mechanism of protein biosynthesis taken in consideration it seems probable that the methylated amino acids do not take part in the biosynthesis of methylated histones.) For this vary reason, in spite of the fact that the tumour stimulation of methylated amino acids has been demonstrated, we do not believe them to fulfil Szent-Györgyi's original assumption of taking part in the *in vivo* cell regulation; we think they only represent its final result.

Of the authors of the paper Tyihák made previous investigations into the biological role of methylated basic amino acids when pointing out a substance of red beet inhibiting tumour formation. It followed naturally from these studies to raise the problem of similarity between the mentioned amino acids on one hand, and "promine" and "retine" on the other. By raising the question neglected for years he gives an impetus to further studies to be performed on the regulatory system presumed in the cells. Biological examinations may later decide whether $N^{G,G}$-dimethyl-arginine really shows a "retine" action.

M. Halmos, K. Kovács, B. Penke, J. Szabó
József Attila University,
Department of Organic Chemistry,
Szeged

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ARE "PROMINE" AND "RETINE" THE METHYLAMINO ACIDS, N^{ε}-TRIMETHYLLYSINE AND GUANIDINO-N-DIMETHYLARGININES, RESPECTIVELY?

In the preparation of biologically active substances from calf thymus, Szent-Györgyi *et al.* (1962) found some fractions which promote or inhibit the growth of ascites tumour, and named the growth-promoting substance "promine" and the growth retarding substance "retine", respectively. This interesting observation drove many investigators to find what "promine" and "retine" are, especially the latter compound, because of the hope that "retine" may be used in cancer therapy. Later, an occurrence of the growth inhibitor and its antagonist was shown not only in the thymus but in various mammalian tissues Szent-Györgyi *et al.* (1963a), human urine Hegyeli *et al.* (1963) and clams Schmeer (1964). Information on the chemical characteristics of "retine" also accumulated (Hegyeli *et al.* 1963, Együd 1965, MARMASSE 1966) and suggested that "retine" is a derivative of methylglyoxy SZENT-GYÖRGYI (1967). From these observations SZENT-GYÖRGYI *et al.* (1963b) proposed the autobiotics concept that the natural substances, "retine" and "promine", regulate the cell growth in organs.

Recently a very interesting finding regarding the identification of "promine" and "retine" was reported by TYIHÁK—PATTHY (1973). They identified "promine" and "retine" with N^{ε}-trimethyllysine and guanidino-N-dimethylarginines on the bases of the method of preparation and some biological investigations. I wish to comment on their papers, especially their identification experiments. However, I have to mention that there are some imprecise comments because some of their papers are not available in Japan.

1. On the preparation. There are a couple of questions regarding the method of preparation for the identity of the samples of Tyihák and Patthy with those of SZENT-GYÖRGYI *et al.* (1962), HEGYELI *et al.* (1963). The latter investigators used chloroform extract for the preparation of "promine" and "retine". They mentioned that the biologically active substances can dissolve in acetone and chloroform. If "promine" and "retine" are the methylamino acids pointed out by Tyihák and Patthy the solubility is very contradictory because of the insolubility of the methylamino acids in chloroform. Another inconsistent point is that "retine" can be distilled at pH 11 by steam and extracted at pH 1.5 into chloroform (MARMASSE 1966). From the above conflicting observation the specific biological activity should be checked at each preparative step for purification although both "promine" and "retine" exist concomitantly. It has been known that these substances can be separated by paper chromatography (SZENT-GYÖRGYI *et al.* 1962). Therefore, the biological activities of the fraction of each step should be estimated after the separation of the "promine" and "retine" fractions.

2. On identification of the methylamino acids. It is inconsequent to use very crude materials for the identification experiments. The data shown by Tyihák and Patthy only suggested that their samples may contain the methylamino acids. It is required to purify the sample till it contains only a single spot before use for an identification study. To confirm the identification it is also necessary to make some identified derivatives of the compounds chemically of enzymatically unless information of their elementary analyses, infrared spectra etc. are available. For reference nuclear magnetic resonance spectra, their interpretation, R_f values and migration distances of the methylamino acids are presented in Fig. 1, Tables 1 and 2.

3. On the identification of the methylamino acids with "promine" and "retine". It is incorrect that the conclusion was made from the short circuited linkage of the pharmacological effects (SZENDE *et al.* 1970, KOPPER *et al.* 1971) of the methylamino acids and their occurrence in the crude preparative samples. There are many identified and unidentified compounds in the samples. Therefore, it is necessary to determine mainly which fraction or compound recovers the biological activities. In general, portions of lower R_f values in paper or thin layer chromatography contain more compounds than those of higher R_f values. Because of inavailability of information on the specific biological activities of the methylamino acids prepared from the materials a clear-cut comment about their identities with "promine" and "retine" cannot be presented. However, it should be noticed that a very low concentration of these methylated amino acids occur in mammalian organs as free forms except in urine. Therefore, I recommend using human urine (KAKIMOTO—AKAZAWA 1970) or hydrolysate of protern (NAKAJIMA *et al.* 1971) rich in the methylamino acids for a definite conclusion on the identification of "promine" and "retine". If the methylated amino acids are "promine" and "retine", it remains to be resolved that these methylated compounds really regulate the cell growth in situ as mentioned by Szent-Györgyi because of their very low concentrations in organs in free forms.

Any way the interesting findings reported by Tyihák and Patthy give a clue to the autobiotics concept of Szent-Györgyi and an elucidation of the physiological significance of

Table 1

Interpretation of nuclear magnetic resonance spectra of guanidio-N-methylarginines

N^G,N^G-Dimethylarginine Chemical shift (τ) structure	7.75 (4H) $C-CH_2-C$	6.80 (6H) $N^G\langle\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$	6.48 (2H) $N-CH_2-C$
N^G,N'^G-Dimethylarginine Chemical shift (τ) structure	7.60 (4H) $C-CH_2-C$	6.93 (6H) 6.83 N^G-CH_3 N'^G-CH_3	6.36 (2H) $N-CH_2-C$
N^G-Monomethylarginine Chemical shift (τ) structure	7.80 (4H) $C-CH_2-C$	7.00 (3H) N^G-CH_3	6.58 (2H) $N-CH_2-C$

N^G,N^G-Dimethylarginine Chemical shift (τ) structure	5.40 (1H) $N^\alpha-CH-C$	3.90 (2H) $NH=C-NH$	2.30 (2H) $\alpha-NH$
N^G,N'^G-Dimethylarginine Chemical shift (τ) structure	5.20 (1H) $N^\alpha-CH-C$	3.92 (2H) 3.82 $C-NH-C-$ $NH-C\ \|$ N	2.26 (2H) $\alpha-NH_2$
N^G-Monomethylarginine Chemical shift (τ) structure	5.50 (1H) $N^\alpha-CH-C$	3.78 (3H) $NH-C-NH_2$	2.32 (3H) $\alpha-NH_3^+$

Table 2

R_f values and migration distances of the methylamino acids

Paper chromatography: solvent #1=pyridine-acetone-3M NH_4OH (50 : 30 : 25); #2=isopropanol-formic acid-water (4 : 1 : 1); #3=n-butanol-acetic acid-water (4 : 1 : 1); #4=n-butanol-pyridine-acetic acid-water (4 : 1 : 1 : 2). High voltage paper electrophoresis was carried out in a mixture of pyridine-acetic acid-water (5 : 50 : 945, pH 3.4) at a potential gradient of 100 volts/cm for 30 minutes. The compounds were visualized with ninhydrin

Compounds	R_f values				Migration distance (cm)
	#1	#2	#3	#4	
Lysine	0.46	0.46	0.14	0.19	20.9
N^ε-Monomethyllysine	0.40	0.57	0.18	0.23	19.8
N^ε-Dimethyllysine	0.58	0.61	0.16	0.23	19.5
N^ε-Trimethyllysine	0.17	0.59	0.16	0.20	19.8
Arginine	0.20	0.52	0.19	0.26	19.8
N^G-Monomethylarginine	0.30	0.64	0.24	0.29	18.9
N^G,N^G-Dimethylarginine	0.34	0.66	0.26	0.29	18.1
N^G,N'^G-Dimethylarginine	0.42	0.68	0.28	0.32	17.4

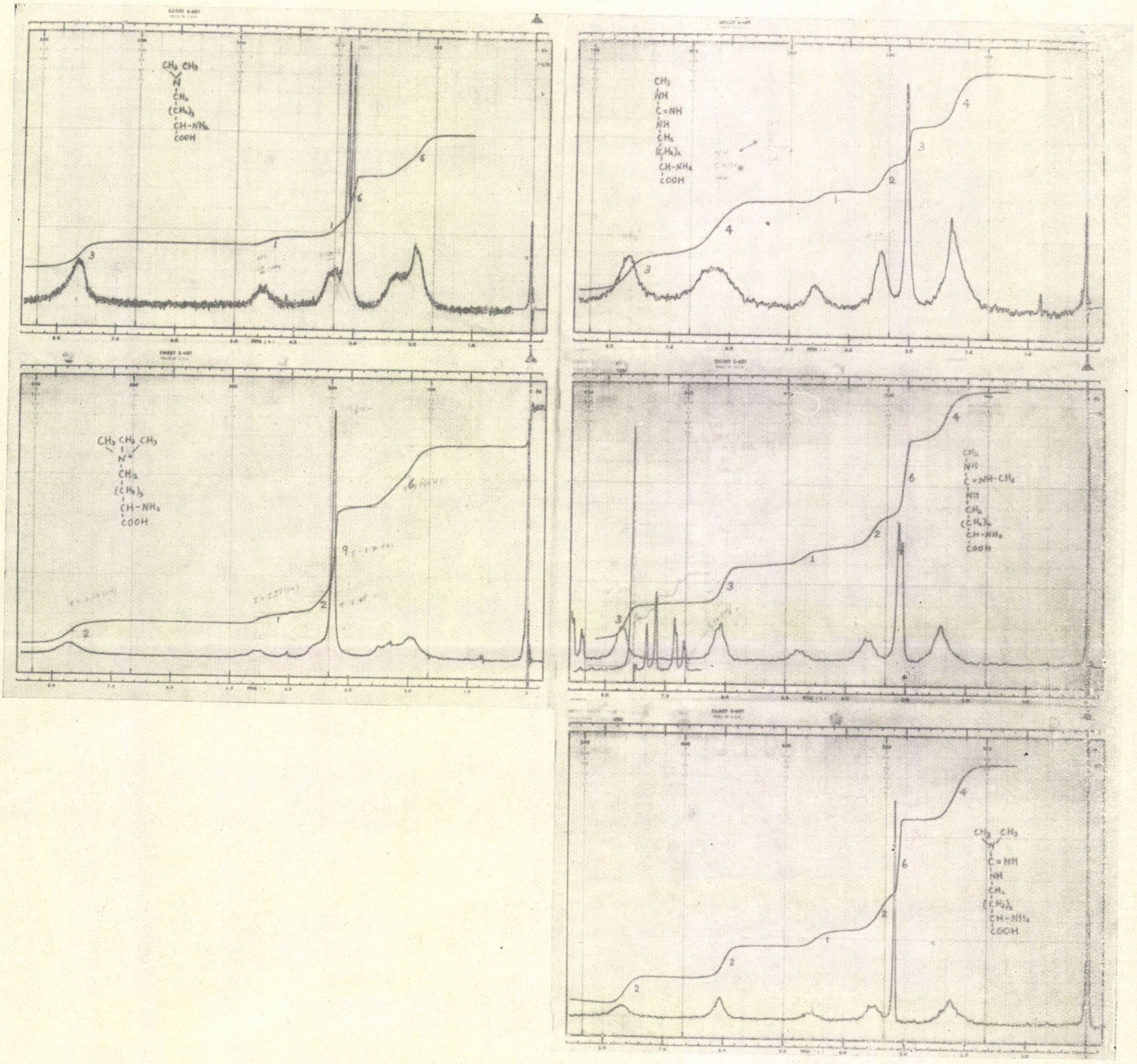

Fig. 1. Nuclear magnetic resonance spectra of the methylated lysines and the methylated arginines in trifluoroacetic acid using tetramethylsilane as inner standard substance

methylated amino acids. Our group first thought of three possibilities regarding the physiological significance of N^{ε}-methylated lysine and guanidino-N-methylated arginine residues in proteins; the modification of proteins like acetylation, phosphorylation and adenylation which are related to the regulation of enzyme activity, a signal of the ageing of proteins and essential constituents of proteins like hydroxyproline and δ-hydroxylysine in collagen. The first two possibilities are not probable because of the same turnover rate of the methylated amino acid residues and proteins in tissues and because of more resistance of the chemically methylate proteins to proteinases. Therefore, we now have a third hypothesis that some proteins synthetized in the cells are subjected to methylation in their arginine or lysine residues to establish their mature structure and configuration. As for the physiological significance of the free methylated amino acids we have thought that they are only endometabolites of proteins since large amounts of the methylated amino acids are excreted into urine without reabsorption in renal tubules. The finding of Tyihák et al. is, therefore, very interesting for us although there are many problems to be elucidated, namely mechanisms of the regulation of cell growth by the methylated amino acids, etc.

Teruo Nakajima
The Department of Neurology
The Institute of Higher Nervous Activity
Osaka University Medical School
Fukushima-ku, Osaka, Japan

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IN WHICH PHASE OF THE CELL CYCLE DO THE METHYLIZED FORMS OF ARGININE AND LYSINE EXERCISE THEIR SPECIFIC STIMULATORY OR INHIBITORY EFFECTS ON CELL DIVISION?

Investigations into the causes of malignant tumour formation have long been in close connection — both from a theoretical and a practical aspect — with cytological studies on cell division and its exciting agents or inhibitory factors, and on the related physiological and biochemical questions. In their studies the authors isolated and chemically defined two substances which — according to the evidence of biological experiments — had a considerable inhibitory or stimulatory effect on tumour formation. The results are all the more remarkable because the compounds concerned are amino acids, certain methylized forms of arginine and lysine, that is, substances occurring in the cells under natural conditions too.

What makes the authors' study especially timely is that today the protein synthesis related with this process is a central problem of the chemistry of cell division. From a cytological point of view the earlier autoradiographic examinations performed with radioactive amino acids which showed a highly intensive protein synthesis occurring in the interphase when DNA was replicated, and taking place in the prophase too, were especially convincing. According to the autodiagrams this process takes place both in the cytoplasm and the nucleus. In the nucleus histone formation is the domineering process which has a highly important role in the structure of the chromosome and probably in the regulation of the gene activity too. This circumstance greatly increases the importance of the authors' work. It is already known that there is a close time correlation between the DNA and histone synthesis, as proved by the microphotometric, autoradiographic and biochemical analyses alike. It seems that the larger part of the histobe synthesis slightly precedes the DNA synthesis. As regards the meiosis, it was pointed out that different proteins were synthetized in the individual phases. This change is especially interesting at the zygote stage where the process of chromosome coupling is supposed in the first place. This was proved by the fact that the normal course of meiosis could be prevented by protein synthesis inhibitors. In this context it can be said in general, that from the point of view of clarifying the action mechanism of factors stimulating or inhibiting the division of cells it is highly important to find out in which, or on which phase of the cell cycle they act. Namely, today it is quite clear that cell division is only a part of the full cell cycle, although it involves highly significant morphological changes. On the other hand, processes preparing the cell division, which are of a biochemical rather than structural nature, take place in the interphase. Hence, with all probability a chemical agent, be it an amino acid, exerts its specific stimulatory or inhibitory effect on cell division, and thereby on the further course of the cell cycle, in a definite phase of the cell cycle.

L. Fridvalszky
Eötvös Loránd University; Department of
Applied Botany and Histogenesis
1088 Budapest, Múzeum krt. 4/a.

CAN PROMINE AND RETINE BE FOUND IN TOBACCO GENETIC TUMOURS?

Tumours have an increased mitotic activity. Cell division is regulated by the stimulatory and inhibitory substances of cells and their relative proportion determines the normal or the tumours development (Szent-Györgyi 1965, Szent-Györgyi *et al.* 1967). These two regulatory substances are promine and retine. The exact chemical identification of promine and retine has not been accomplished so far. According to the earlier results diketo-compounds or their derivatives containing nitrogen, glyoxale derivatives, glyoxalase etc. may take part in the promine-retine regulation system.

Tyihák—Patthy (1973), in their paper, declared the success of the final identification of the chemical nature of promine and retine. According to them promine and retine are methylated basic amino acids.

Methylated basic amino acids are important components of basic proteins, histones. Thus, it is imaginable that in tissues free methylated basic amino acids can appear due to the decomposition of histones. (Namely, methylation of the amino acids takes place in the whole histone molecule.) The question is whether the amount of the methylated basic amino acids reaches a regulative concentration in the tissues or not.

The regulative role of histones has been described earlier. In tumour forming tobacco hybrids a close correlation was demonstrated between the histone/DNA ratio and the intensity of protein and nucleic acid synthesis (Kovács 1971a, b, c). On the basis of these experiments the histones would directly regulate the DNA synthesis rather than the protein synthesis (the latter would be indirectly influenced by the histones) (Kovács 1971a, b, c). Thus, it is imaginable that histones could regulate mitosis (M) influencing the DNA synthesis. Mitosis can only start after passing through the period of DNA synthesis (S-phase) of the mitotic cycle. Hence, the DNA synthesis is influenced by histones regulating the mitotic activity, too (Fig. 1).

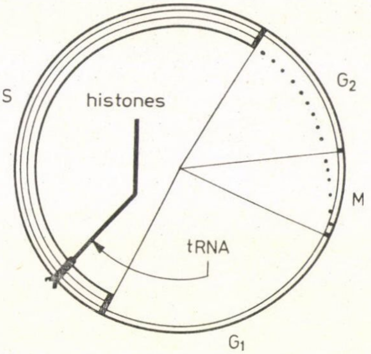

Fig. 1. Regulation of mitosis (M) by histones and tRNA (A possible model. Explanation is in the text.) >— inhibition, ← influence.

In tobacco genetic tumours and in their tissue cultures the complexing of histones to DNA is regulated by the RNA content of the cells (Kovács 1971a, b, c). It follows that the RNA content of the cells could regulate the mitotic activity by influencing the complex formation between the DNA and the histones (Fig. 1).

In recent experiments I have determined the nature of the RNA in the DNA—RNA-histone complex. In the experiments the tissue cultures of *Nicotiana glanca* × *N. langdorffii* tumour forming F_1 hybrid were used. A crude chromatin preparation containing the DNA—RNA-histone complex (Kovács 1971a, c) was extracted by a phenole method (Vold—Sypherd 1968) to extract the nucleic acids. A methylated albumine kieselguhr (MAK) column chromatographic method of Mandell—Hershey (1960) was used to separate the nucleic acids.

The tissue cultures contain transfer RNA (tRNA), DNA and ribosomal RNA (rRNA), respectively (Fig. 2a). The DNA-RNA-histone complex only contains tRNA and DNA but no rRNA (Fig. 2b). Thus, my experiments clearly show the presence of tRNA in the DNA-

RNA-histone complex of the crude chromatin preparation. No ribosomal RNA could be observed in the crude chromatin. These experiments suggest an important regulative role of tRNA in the complexing of histones to DNA and probably in the regulation of mitosis (Fig. 1).

It is probable that the above mentioned regulating role of macromolecules can be influenced by substances of low molecular weight or some regulators of low molecular weight can affect DNA synthesis as well as mitosis by binding to macromolecules. These are suggested by several experiments.

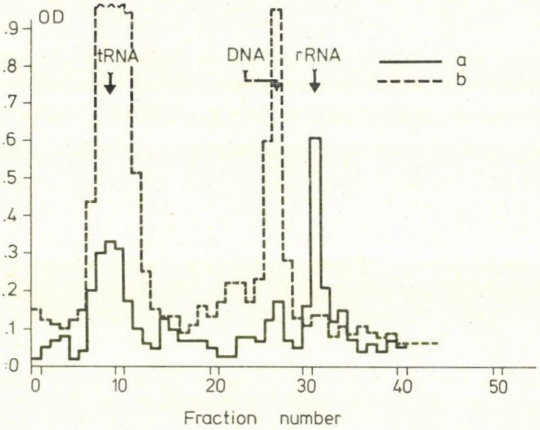

Fig. 2. MAK column chromatographic profile of nucleic acids of the tumourous tissue cultures (a) and of the crude chromatin preparation (b) derived from the tissue cultures. Nucleic acids of six g of the tissue culture (a) and chromatin of 22 g of the tissue culture (b) were used for chromatography

Earlier, it was experienced that a medium containing starch inhibits the characteristic growth and development of tobacco tissue cultures of genetic tumourous condition (Kovács 1970). The antitumour effect of starch has not been known so far. The nucleic acid and protein content of these tumourous tissue cultures becomes lower (inhibition). The starch medium may bring about a reduced energy supply or, on the other hand, some unknown decomposition product of starch (not glucose or maltose) can influence the nucleic acid and protein synthesis affecting the growth and organization of tumourous tissue cultures (Kovács 1970).

The above mentioned concepts are supported by results of other researchers. The antitumour activity of different polysaccharides was observed by Chihara *et al.* (1969) and Sasaki *et al.* (1970).

The regulative role of polysaccharides is reflected in synchronous culture experiments showing an inverse change between the mitotic activity and starch content of cells (Cameron—Padilla 1966).

The results clearly show the presence of stimulatory and inhibitory factors of cell division in tobacco genetic tumours. To know the chemical nature of promine and retine like substances of plant genetic tumors requires further examinations.

E. I. Kovács
Department Genetics,
Eötvös Loránd University,
1088 Budapest,
Múzeum krt 4/a.

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CAN THE PROCESS OF TUMOUR FORMATION BE INFLUENCED BY EITHER ADDING OR LEAVING OUT SIMPLE PROTEINS OR AMINO ACID DERIVATIVES?

The authors deal with the chemical identification of promine and retine. They unequivocally state that the substance called promine by SZENT-GYÖRGYI—HEGYELI (Biol. Bull., *123*, 466, 1972) — which has a stimulatory effect on cell division — corresponds to methyllysines, while retine — an inhibitor of cell division — to methylarginines. They support the chemical identification with literary data and their own experiments.

In the experiments of Szent-Györgyi and his collaborators both substances proved to be dialysable and of less than 1000 molecular weight. Both promine and retine are compounds containing nitrogen, have some tendency to dissociation and — accordingly — can be separated with Reinecke salt in 2—3 n HCl, in the same way as choline and the bétain type compounds.

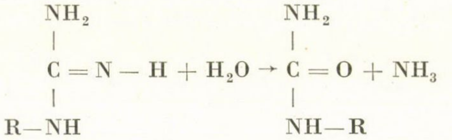
One of the authors — Tyihák — has been dealing with the methylated derivatives of the three basic amino acids (Arg, His, Lys) since 1964, and in the course of separation by paper chromatography found the lysine and arginine derivatives to be of the same bahaviour as promine and retine.

From a different aspect — when studying bétain type compounds — Tyihák had earlier recognized their inhibitory effect on tumour formation, and this was enough to turn his attention to the transmethylation processes and the methylated amino acids.

Tyihák — collaborating with researchers expert in the metabolism of tumour formation — points out that all three methyl derivatives of lysine stimulate the growth of normal tissue in vitro, and that of tumourous tissues in vivo. During the past nine years the author proved in a few shorter, and one major comprehensive publication that the methylated amino acids are not restricted to the thymus but are spread in a wide range of living organisms both in a free state and as incorporated in proteins. That is why he has set the aim of investigating the general biological and the biochemical properties of methylated amino acids.

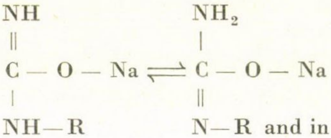
As a result of his work, in co-operation with one of his collaborators he has elaborated and patented a method for the production of tumour inhibiting basic proteins.

He did not let himself follow any side-track-like Szent-Györgyi's line showing so many positive features and diversity in the original objectives — but proceeded consciously and deliberately towards his aim. The author's present work — like his earlier papers — is in an analytical sense an extremely careful and thorough study. He succeeded in pointing out without any doubt whatever that both ε-N-methylated lysines, and arginines methylated in their guanidine group occur in the thymus, but — as he himself writes on page 11 — the presence of other substances too must be reckoned with in the thymus fractions (BT-II), and as for their biological activity no experiment has been carried out so far. In addition, the author has neither literary data, nor experimental evidence of the activity of methylated arginines, or even of their identity with retine present in the thymus. The demonstration of the retine character of the compounds in question would have been desirable, as this has remained a weak point of Szent-Györgyi's line too. The isolation and identification experiments only outlined the above compounds as being of keton- and methylglioxale nature, but could not identify them, as referred to in the authors' paper too. The chemical identification of methylarginines obtained from biological objects is thus due to Tyihák. It is true that through the reaction of the imino group in its guanidine group arginine is able to part with it and produce the carbamidyl group



$$\begin{array}{c} NH_2 \\ | \\ C=N-H \\ | \\ R-NH \end{array} + H_2O \rightarrow \begin{array}{c} NH_2 \\ | \\ C=O \\ | \\ NH-R \end{array} + NH_3$$

and in an alkaline medium both isomer enolate forms may even occur:



$$\begin{array}{l} NH \\ \| \\ C-O-Na \\ | \\ NH-R \end{array} \rightleftharpoons \begin{array}{l} NH_2 \\ | \\ C-O-Na \\ \| \\ N-R \end{array}$$

and in this way it may show in its properties the criteria established for retine. Statements of that kind may have led to the idea of the glucosulose-3-deoxy derivative and dehydro-ascorbic acid and many other compounds not mentioned here — besides the glioxale derivatives — being identical with tumour inhibitors; further, the researchers were stimulated to try out a number of synthetized products with the view of their possible utilization in chemotherapy. The authors have not presented evidence of the biological activity so far, so we can only rely on the remark found in the paper: "the retine character of guanidino-methylated arginine derivatives will be decided by the biological examinations in process."

Due to their promine-like effect stimulating proliferation and influencing tumour growth, the chemical identification seems to be more unambigous in the case of the methyllysines than in that of the arginine derivatives. But even here we must be careful with the statement of a general promine-like effect, since there are numerous cytostatic compounds interfering with the ontogenetic phases of cells. These compounds (chalons Bullough, W. S, 1971 Nature *229*, 608 and Nature New Biology *231*, 862 1971, editorial paper) have many types (low and high molecular weight proteins, amino acids and other simple compounds) known as agents of mitodepressive or mitogenic action. In my opinion lysine or arginine methylated in various degrees may be one of these compounds, as suggested by one of Tyihák's collaborators too (KOPPER *et al.* 1971 Neoplazma *18*, 60) in connection with their action in starting DNA synthesis.

When comparing the present publication of Tyihák and his collaborators as well as their earlier activities with what have been said above, the evidences given seem to be insufficient, namely, the authors describe the identity of retine and promine from one aspect only. Accordingly, they think the compounds in question to act in a relatively simple (chemical) way. Although Tyihák has recognized that the compounds identified by him are products of enzymatic processes — the activity of methyl-transferases —, they are not yet related with definite metabolic processes where the way of their biological action would be shown.

I disagree with the authors' opinion of the methylated amino acids being decomposition products of the corresponding proteins. I am sure that both the free amino acids and the methylated basic amino acids occurring in proteins have separate biological functions too. It is clear also from the data of TYIHÁK's summarizing publication (Magyar Kémikusok Lapja XVII No 11 1972 and J. Jap. Biochem. Soc. *44*, 353, 364) that free methylated derivatives occur even in the absence of histone or other proteins supposed to contain them. On the other hand, I consider the process of tumour formation to involve changes too intensive to be influenced by either adding or leaving out amino acid derivatives. Namely I regard the cessation of cell regulation and formation of proliferating tissues as changes of ontogenetic and phylogenetic nature. When the G_1 phase — that means the period of specialization — is left out from or shortened in the life of the cell, the phylogenetically determined life of cells — which are thus characterized in each tissue and organ — ceases, and only the phases of growth and division alternate in the proliferating cells. Cessation does not happen in an instant either; it is a long process in which the cells fight for their historically developed and regulated metabolism (this state is known as precancerous or pretumourous stage). In this state some compounds indicating and characterizing the process may appear, but in my opinion they are not uniform like retine and promine, but according to the metabolism of tissues are differentiated, in the same way as the masses of proliferating cells deprived of the G_1 phase vary according to their origin and the changes occurring in their metabolism. However, the possibility of promine, retine or methylated amino acids playing such an important role is not excluded.

In spite of the comments made above I attach great importance to this work, as it proves that many a step unavoidable in investigating cell division and tumour formation has been taken in this field. It has opened a new phase of the research of retine, promine and proliferation, and may even start a new chapter in the therapy of proliferating tissues.

S. FAZEKAS
Semmelweis Medical University
Department of Biochemistry
1088 Budapest

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CHRONICA

ZOLTÁN ZSÁK

(1880—1966)

It was on October 13th, 1966, at the age of 87, that Zoltán Zsák, the internationally reknown Hungarian expert of seeds and researcher of Hungarian flora died. With his death his colleagues and friends lost a cheerful companion always ready to help and teach.

Zoltán Zsák was born at Nyíregyháza on February 3rd, 1880. He was the second of five children. His parents were Endre Zsák, Lutheran schoolmaster and cantor, and Emilia Nádassy. His father soon recognized the importance of learning and knowing foreign languages and sent his son Zoltán to a North-Hungarian region inhabited by a German speaking population to acquire a thorough knowledge of German. He attended school there except the last year of the secondary school which he completed — and sat for a final examination — in his home town, in 1898.

Being interested in natural sciences the young Zoltán Zsák was matriculated at the Faculty of Mathematics and Natural Sciences of the Kolozsvár University in the same year. He became especially interested in botany and joined in the work of the Department of Botany under the leadership of Prof. Aladár Richter, where in January 1902 he was also employed. At that time an important change occurred in the life of the University; at the end of the year Vince Borbás was appointed professor of Taxonomy and Plant Geography, and the Department of Botany was divided into two. Zoltán Zsák worked as assistant to Borbás until the latter's death in 1905, when the two Departments were reunited under the leadership of Prof. Richter. Thus, Zoltán Zsák was pupil and collaborator of both of them.

One of Prof. Richter's large-scale projects was to establish the largest possible herbarium at Kolozsvár. For this purpose he collected a vast material, and made his collaborators do the same. So, at both Departments — as assistant to Prof. Borbás or to Prof. Richter — Zoltán Zsák had an opportunity to roam about large areas and collect vast material, which increased his knowledge of plants to a great extent. Zoltán Zsák had extremely keen eyes to notice the slightest differences; this faculty was a great help in his later activity. In 1904 in the gorge of Torda (Transylvania) he discovered a very interesting *Valerianella* which proved to be a so far unknown hybrid of *dentata* × *rimosa* and was named by Vince Borbás *Valerianella Zoltáni* after its discoverer.

In the same year he published his observations on those specimens of the family *Fumariaceae* which differ from the other completely glabrous members in being hairy. These observations were also due to his extremely keen eyes.

These results called the attention of Árpád Degen, director of the Seed Testing Station of Budapest to Zoltán Zsák who was invited in 1908 to work at the institute. Árpád Degen wanted to employ the highest possible number of experts to ensure the efficient work of the institute and a possibility for giving correct answers even in the most particular problems.

Zoltán Zsák accepted the invitation and entered service at the Seed Testing Station of Budapest on November 8th, 1908. He had more than one reason for doing this. From a human point of view, the post meant a safe existence after his uncertain employment at the University; from a professional point of view, working in Degen's institute meant — beside his occupation of agricultural botany and seed testing — the continuation of his taxonomic studies and researches of the Hungarian flora, since Árpád Degen was then the internationally acknowledged Hungarian leader of these two scientific lines excluded at that time from the University.

Zoltán Zsák began his work with great enthusiasm. He learned the methods of seed testing, got acquainted with the problems and — in the meantime — continually improved his knowledge of plants indispensable in this work. He made regular excursions all over the country, collected plants and seeds, so under systematic and expert guidance he acquired a wide knowledge of plants and seeds in a relatively short time.

He dedicated all his subsequent life to his profession and worked almost all the time at the Institute for Seed Testing.

For one and a half years, during 1911—1913 he was employed at the Hungarian Institute for Seed Improvement at Temesvár, and was leader of the Seed Testing Institute at Kassa from January 1941 to November 1943. Apart from these two intervals he always worked in Budapest.

His first years of service were spent in collecting plants and seeds. It often happened that in one of the seed samples he found the seed of an interesting or rare weed, and searched the respective area for the plant itself to make certain of the correctness of his definition.

In 1915 and 1916 he reported on a number of interesting botanical discoveries and, in addition, studied hybrids — especially in the genera *Inula* and *Cirsium* — with great interest. These studies resulted in a doctoral dissertation submitted in 1920, on the basis of which he took a doctor's degree in humanities with the highest praise.

The Institute for Seed Testing issued 2 herbaria for practical purposes; one of then was a "Collection of Hungarian *Gramineae*", the other the "Collection of Hungarian *Cyperaceae, Juncaceae, Typhaceae, Sparganiaceae*". The aim was to promote the management of pastures and meadows in Hungary, but both collections were also of high scientific value. Zoltán Zsák took an important part in the work of collecting material mainly as a companion of Árpád Degen. Nevertheless, his name can also be found among the collectors of a herbarium of theoretical importance edited by the Botanical Collection of the National Museum.

His extensive practical and theoretical knowledge was utilized in other fields too. For example, he joined in the preventive work of the Entomological Station in three successive years at the time of the Maroccan invasion of locusts; took part in the potato disease control of the Phytopathological Station; supervised hay analyses carried out for the Zoobotanical and Feeding Station; delivered lectures on apicultural botany at a course of the Association of Apiculturists. In 1926 the Institute of Geology carried out a nation-wide survey of alkali soils with several committees employed. Zoltán Zsák participated in the work as a member of committee No. 6. When studying the economic importance of pheasants, quails and partridges the Institute of Ornithology asked Zoltán Zsák to do the work of seed testing.

With the collaboration of Guido Gerhardt Zoltán Zsák compiled a collection in 1936 entitled "A magyar búza gyommagvai" (Weed seeds of Hungarian wheats) containing 120 various seeds, and another one in 1943 under the title "A magyar lóhere és lucerna fontosabb gyommagvai" (Major weed seeds of Hungarian clover and alfalfa) containing 240 seeds in 2 boxes. From 1953 on he issued — in common with András Barthodeiszky — the "Gyommaggyűjtemény" (Collection of weed seeds) in 4 boxes. These collections were of high importance from a practical point of view.

In 1945 he retired, but this event did not cause any essential change in his activity, as the Institute — which in the meantime had developed into the National Inspectorate of Seeds — continued to employ him, and he worked there as an expert until his death.

In 1947 he examined grain crops transported to the Soviet Union; in the next year supervised maize- and oat transports sent to Poland at the border station of Szob; in 1949 he delivered lectures on papilionaceous fodder plants and their seeds at the party school of Békéscsaba as commissioned by the educational department of the National Co-operative Centre where within the framework of individual occupation he gave his working system over to 50 participants. In 1951, upon the request of the Rector of the University of Agricultural Sciences he prepared a sufficient amount of the most important seeds for the purpose of teaching about weeds. He compiled an extra collection of the seeds of 250 species for the Department of Soil Cultivation.

His wide knowledge was also utilized in other branches of science. For example, at the request of the National Historical Museum he identified about 2500 g of carbonized seeds of the Bronze Age found during excavations in the neighbourhood of Nagyárpád, county Somogy. The results were published in 1959 as his latest work. He added the identifications of several minor findings to others in order to publish them; these were presented in the 1967—68 publications of the Hungarian Agricultural Museum. The Museum of Applied Arts gave him a Tibetan wizard's apron for examination and he recognised the big shiny fruits of *Coix lacryma-jobi* in it.

This many-sided activity well reflects his extensive knowledge to which only his infinite modesty was superior. He never wanted to acquire any title or decoration, even the results of his studies, researches and investigations were published only on other people's encouragement.

The fact that 6 flowering plants, 1 mushroom and 1 lichen were named after him proves his colleagues' respect for him but there was no lack of official appreciation either. In 1943 he was acknowledged by the Minister, then conferred the title and rights of director-general of research work. In 1950 he was rewarded by the Hungarian Academy of Sciences, in 1956 became an "Outstanding worker", and in 1958 was decorated with the Medal of Merits in Socialist Work. The Committee of Scientific Qualification declared him in 1962 "candidate in agricultural sciences".

In his private life besides joys he was visited also by terrible blows. Having taken up post at the Seed Testing Station, and his existence thus becoming safe, he married in 1911. From his marriage with Irma Kaszanichky 3 children, 2 girls and a boy, were born. In 1923, when their youngest child was not even 1 year old, he lost his wife. Zoltán Zsák widowed with 3 children looked for a companion, a mother for his children, and married Emma Göllner. He had to suffer, however, another great misfortune in 1963, when his second wife died.

Zoltán Zsák — thus left a widower the second time — found home in the family of his daughter Olga, wife of Zoltán Fejérváry. It was here he lived during his last years, and here he died suddenly.

In his colleagues' and friends' recollection he continues to live as a serene, kind, lovable man who readily shared his great knowledge with those who turned to him.

His memory will be kept alive by his works and the love and respect of his colleagues.

† Z. E. KÁRPÁTI

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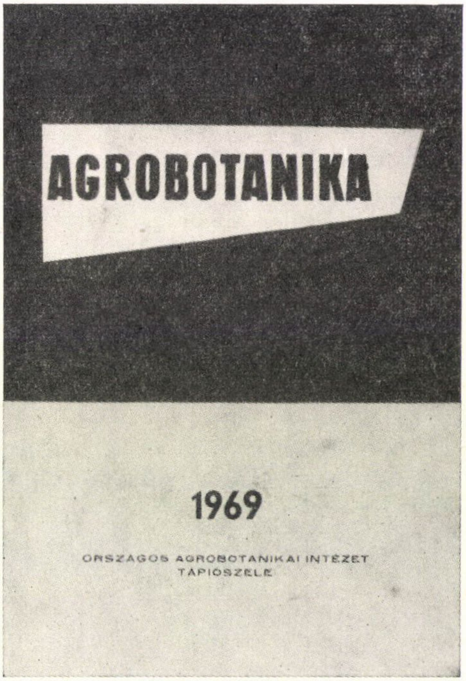
RECENSIONES

Agrobotanika. Tápiószele 1971. 223 pages, 46 tables, 37 figures.


AGROBOTANIKA

1969

ORSZÁGOS AGROBOTANIKAI INTÉZET
TÁPIÓSZELE

The XIth volume of "Agrobotanika" published annually by the National Institute of Agrobotany, Tápiószele, contains 20 papers written by the research workers of the Institute. The volume is completed by the short English summaries of two doctoral dissertations. The papers give a true picture of the many-sided work done by the Institute not only to Hungarian readers, because the English summaries of the papers, the captions of diagrams and pictures written in two languages give adequate information to foreign readers too, and the introduction of the volume — in which under the heading "10 years of the Institute of Agrobotany" A. Jánossy, director of the Institute summarizes the ten years history, many-sided work and great progress of the Institute — is fully published in three world languages. (A full list of 192 papers published by the research workers of the Institute during ten years is contained in the Xth Volume of "Agrobotanika".)

The papers discuss the most diversified details of agrobotanical research. A brief paper by Á. Bárdy: "Classification of Hungarian local maize varieties" describes — as a preliminary publication — 5 local varieties of identical grain and cob colour from each of three maturity groups formed within each of the dent corn and flint corn convarieties, and finds considerable differences between the local varieties even within the same group.

L. Bányay: "Agrobotanical study of millet varieties." The author reports on studies performed with 99 Hungarian and foreign millet varieties. Data of a total of 17 morphological and phenological surveys of the varieties examined during the two years of the experiment are published. On the basis of a detailed analysis of the data conclusions are drawn as to the production value of the varieties too.

L. Márkus et al. studied the intensity of photosynthetic carbon dioxide fixation at temperatures of +21°C, —0.5°C and —1.5°C to characterize the frost tolerance of intensive wheat varieties. In frost resistant varieties intensity was reduced by low temperatures to a much lower extent.

Gy. Mándy and L. Szabó: "Results of ecological studies on CCC-treated wheats." On the basis of experiments carried out in 1966 and 1967 the authors found that the effect of CCC treatments highly depends on the wheather.

L. Heszky with his paper on the causes of automatic dehiscence in the lucerne flower completes his study published in the previous, Xth volume of "Agrobotanika" on the closing and opening mechanism of the lucerne flower. The repeated presentation of text and photos required for comprehension ensures that the paper is valuable even in itself, without the first part. The fully detailed description of the investigations is completed by abundant references to the literature of the subject.

I. Sulyok: "Effect of weather on flowering in the lucerne collection from the second year of use." The paper continues the author's study published in the previous volume on the same subject but with the results of the first year, the year of sowing. The paper presents the results of investigations carried on for seven years.

Á. Kiss jr., in his paper dealing with the structure of the paprika flower describes studies on the position of the stigma to the anther, carried on over two years with 118 foreign and Hungarian red pepper (paprika) varieties. The table of results shows considerable differences between the varieties. Since stigmae surmounting the anthers promote cross pollination, it is with these differences that the author explains the contradictions found in the literature concerning self-pollination in red pepper.

Investigations made by B. Koch et al. into the components of lucerne complete the results published by the authors last year with data obtained in 1968. While emphasizing the excellent potential productivity of lucerne they present the NO_3-, lysine- and methionin contents too.

B. Koch and M. Pintácsi: "Haemolytic saponin studies with some fodder plants." The paper gives the haemolytic saponin contents of 15 various fodder plants with a precise description of a quick method of examination. Very great differences could be found between the individual plant species.

B. Koch et al.: "Crude protein-, lysine- and methionin contents in some Trifolium and Vicia species of the Hungarian flora." When studying 12 and 13 wild species of the two genera respectively, the author found no significant differences between the two genera on the average of the species, except for a somewhat higher protein content in the vetches; between the individual species, however, significant differences were shown, especially in the methionin content of the Vicia species.

B. Koch—Á. Boros—L. Szabó: "Crude protein-, alkaloid-, sterol- and saponin contents in some species of Hungarian flora." The paper is a preliminary report on the results of investigating a total of 92 plant species from 23 families. The highly valuable work done to explore new protein sources of plant origin has already yielded high crude protein content (28%) plant species free of noxious substances (e.g. *Lepidium draba*, *Malva neglecta*).

I. Vinceffy—M. Kota give the detailed chemical analysis of samples collected from 50 grass species, presenting, among others, the nitrate protein-, lysine- and methionin contents besides the crude protein content. Outstanding differences were found especially in the methionin content between the plant species; some grass species proved to be highly valuable. The development stage of the collected samples is not given, so differences between the protein data of the two papers can certainly be explained with the different stages of development. As the paper points out too, final results can be obtained only through further investigations carried out with samples of identical development stage.

L. Szabó studied the effect of gibberellic acid and benzimidazole on germination in wheat and barley, from June 16 throughout maturation and post-maturation, with the two chemicals applied both separately and jointly. The brief but substantial paper gives an account of the successful interruption of the dormancy of seeds: pre-cooling also had a very strong effect in this respect. The results are illustrated by detailed figures easy to survey. The paper does not give any explanation for the possible causes of a considerable decrease in July in the germination of the control and the benzimidazole treatments.

J. Mesch—L. Szabó: "Effect of pre-cooling on germination in 100 winter wheat varieties during post-ripening." The paper reports on significant differences found between the reactions of various wheat varieties. The results of investigations from August 14 to May 12 are presented in figures and tables, although the control reached maximum germination values earlier (Nov.—Dec.) and so no further changes would have been expected. A considerable — supposedly significant — decrease in the germination power of the control shown on October 9 is not explained. Changes in the mean values of the 100 varieties are presented both in tables and figures: this double presentation is unusual, since the data can be read precisely from the well arranged table alone.

Gy. Mándy et al.: "Study of the cardinal points of germination in poppy and flax varieties." The paper remedies a deficiency or uncertainty felt in the literature in this field by presenting the minimum, optimum and maximum germination temperatures of the two plant species as well as the life duration of seeds as determined by germination, on the basis of detailed experiments.

L. Szabó—Á. Szűcs, when studying water uptake by the seeds of dwarf beans, measured the rate of water uptake at three different temperatures in 10 varieties and found characteristic differences between the varieties. The differences were in no correlation with either the thousand-grain-weight or the size of the cotyledon. The results give the optimum temperature of water uptake to be 25°C.

E. Papp, when studying the seeds of some wild tomato species presents — besides their morphological data — the germination temperature requirements of the seeds, comparing them with cultivated varieties. Her studies aimed at determining more reliably the ripening stage of cucumber seeds proved the pH-value of cucumber juice to be a figure indicating the stage of ripening.

I. Vinczeffy: "Study on the seed production of grasses." The first part of the series of papers which dealt with toxic weeds was published in the previous Xth volume of Agrobotanika. In the present volume the second part, which deals with the composites, presents the seed production data of 20 plant species, while the third part those of 6 grass species. The comprehensive work covers a plant material collected from various grassy lowland areas, presenting, besides the seed number, the number of capitula and panicles, respectively, completed, in the case of grass species with the data of the spikelets. It provides information valuable for practical grass-seed production too, especially because the seed production data of non-fertilized grasses are completed with those of fertilized, as well as irrigated and fertilized grasslands.

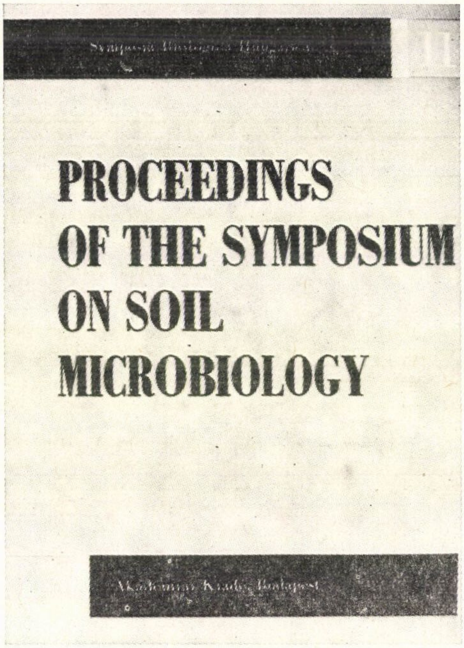
The titles of the two doctoral dissertations summarized in English as an appendix are: I. Sulyok: Correlation of developmental phenomena with weather in lucerne varieties; and L. Szabó: Development of balsam canals and the localization of tannin in some organs of sumac (*Cotinus coggygria* Scop.).

The papers published in the volume contain valuable data obtained by original investigations, presenting literary references concerning the part subjects too. Unfortunately, the process of printing takes so much time, that vol. XI. dated 1969, could be published only in 1971; this delay is sometimes disturbing with the references too.

The valuable results published offer useful assistance to everyone interested in the subjects listed.

L. Berzsenyi-Janosits

Proceedings of the symposium on soil microbiology. Symposia Biologica Hungarica. Vol. 11. Akadémiai Kiadó, Budapest 1972.



PROCEEDINGS
OF THE SYMPOSIUM
ON SOIL
MICROBIOLOGY

Volume 11 of Symposia Biologica Hungarica (edited by J. Szegi, co-editor T. Pátkai) contains lectures delivered during the sessions of the International Symposium of Soil Microbiology organized by the Hungarian Academy of Sciences and the Hungarian Society of Agricultural Sciences held 16—20 June, 1970 in Budapest.

66 lectures presented by 97 research workers of 19 different countries were discussed on two major themes: Section I: The role of microorganisms in the transformation of soil organic matter (48 lectures) and Section II: Interactions between herbicides and microorganisms (18 lectures).

The lectures are published in the book in this division on 454 pp (illustrated with 177 figures and 153 tables and with 517 citations).

The Symposium (the book) is the first of its kind dealing together with the old problem of the microbiological decomposition of soil organic matter and intensively with one of the latest ones: pesticide—microbe interaction arisen only in the last 1—2 decades.

In this way the Symposium is one of the first scientific sessions of Europe discussing the results concerning pesticide-soil microbe interactions, at least within the framework of a Section.

As regards the style of the book owing to language barriers (the official languages of the Symposium were English, Russian and Hungarian) and thus the many authors, it is, naturally, not homogeneous.

Neither did it help that sometimes the lectures had to be translated from Russian (or another language) into Hungarian and then to English.

Though this is a nicely got up book, there are some misprints in it, already on the cover and sometimes in the titles etc. too. It could also be mentioned, that the discussions after the lectures were not cited either, presumably because of the size of the book and the system of the series of Symposia Biologica Hungarica.

It should also be noted as a mistake that this book appeared two and half years after the lectures had been delivered at the Symposium.

These, however, do not decrease its scientific value.

It would be rather long to analyse in detail the 66 lectures written in this volume for this reason we only give an outline of the Sections, mainly just mentioning the headlines or titles of some of the lectures.

In the first Section: The role of microorganisms in the transformation of soil organic matter, E. N. Mishustin in his introductory lecture: "Potential and effective soil fertility as related to plant remains" presented a complex review about the effect of straw applied into the soil on the soil microflora, the yield of legumes and grain crops establishing the best possibilities for applying the straw.

The majority of the lectures in the first Section dealt with the microbiological transformation of the plant residues in different soil types and the effect of soil organic matter on the soil microflora and their physiological processes. The above listed problems were also connected in many cases with the effects of the fertilization, cultivation, melioration etc. of soils.

To characterize the many-sidedness of the discussed problems of the first Section a few lectures should be mentioned e.g., Effect of increasing amounts of nitrogen on the microbial transformation of straw in soil (Novák), Lignolitic activity in soils (Mangenot and Reisinger), Role of microorganisms in the decomposition of mosses (Kilbertus), Humification of a ^{14}C-labelled organic matter in soil and the incorporation of ^{15}N humic substances (Freytag and Igel). On the adsorption behaviour of bacteria in the soil (Müller and Hickisch). The role of phylloplane fungi in the early colonization of leaves (Pugh, Buckley and Mulder), Investigations on anaerobic processes in the formation of solonchak and solonetz soils (Vámos), Effects of organic substances on nitrite formation by *Nitrosomonas* (Tandon), On the problem of modelling in soil enzymology (Kozlov et al.) etc.

The Second Section: Interaction between herbicides and microorganisms began with K. H. Domsch's introductory lecture: Interaction of soil microbes and pesticides. The lecture was divided into two parts, namely: Pesticides affecting soil microbes and pesticide degradation by soil microbes.

From the excellent review (with 25 citations) one can get a general survey about the results, research trends of the problem raised in the title of the second Section. The possible effects of pesticides in the soil ecosystem and alternatives for pesticide applications, as well as the influence of pesticides on the oxidation of different carbonaceous substances or the different ways of degradation were illustrated and discussed in the lecture.

In the lectures of Section II the results concerning the effect of herbicides on soil microflora were mainly presented: The action of herbicides on the antibiotical activity of microorganisms (Vlahov and Kamenova, Gousterov and Damyanova, Gousterov, Brankova and Vlahov, Vlahov—Damyanova, Gousterov and Kamenova). The biological activity of certain herbicides on microscopic fungi (Bakalivanov) and the interaction between soil microflora and herbicides: Agelon (Micev and Bubalov), "Casoron" (Nikolova and Bakalivanov), Dalapon and Trichloroacetic acid (Zakharian), Dymid (Mickovski) as well as the effect of certain herbicides on the growth of nitrogen-fixing algae and rice plants (Ibrahim) the behaviour of some herbicides used for weed control in vineyards on different soils (Manninger and Száva) were the interesting lectures delivered. Lectures were also given on: The effect of herbicides on the decomposition of cellulose (Szegi) and Gramoxone on N-fixing microorganisms (Manninger-Bakondi and Takáts) as well as on rhizobium and lupin symbiosis and the grain yield of lupin (Borbély and Kecskés). As regards the sensitivity of rhizobia to herbicides a general survey was also presented (Kecskés).

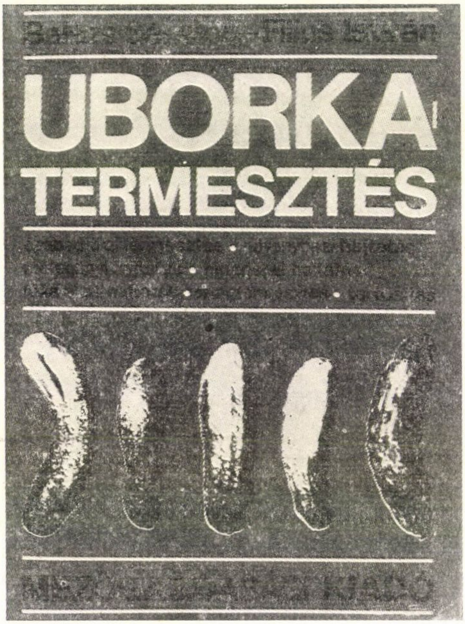
Lectures were delivered dealing with the effect of fungicide seed treatments (Kreaman-Fawaz-Abdel-Ghaffar and El-Gabaly; Elek and Kecskés), fungicide and insecticide treatments (Taha-Mahmoud and Salem) on rhizobia, and rhizobium inoculation.

The proceedings of the lectures presented at the first International Symposium of Hungarian Soil Microbiologists may be of interest not only to soil microbiologists and microbiologists dealing with other aspects of this subject, but also to the specialist working in the different fields of soil science and agricultural practice. As regards the lectures of the second Section dealing with the interaction between pesticides and soil microorganisms they should also be interesting for the specialists aiming at dealing with the protection of the biosphere. The specialized programme of the two Sections gives a possibility of finding some data for holding special lectures at universities too.

Evaluating the Proceedings of the Symposium on Soil Microbiology published as the 11th volume of the Symposia Biologica Hungarica Series, we could establish that it was useful and valuable not only from the point of view of the development of the Hungarian soil microbiological research, but also for soil microbiology as a whole.

M. KECSKÉS

S. BALÁZS—I. FILIUS; *Uborkatermesztés* (Cucumber production). Mezőgazdasági Kiadó, Budapest 1970. pp. 141, 12 tables, 79 figures.



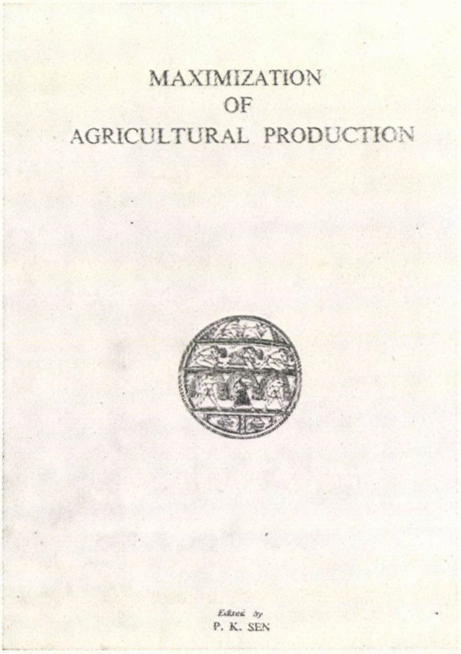
In the series of short monographies on plant production the authors' work was published as a new volume dealing with the field production of cucumber, its forcing in greenhouse, hot-bed, cold-bed and polyvinyl tent as well as seed production and preservation. These short monographies should be considered successful publications as they contain a lot of useful information both for practice and theory in ingenious arrangement.

The book is divided into 9 main chapters and numerous subchapters. In the first chapter the authors introduce the cucumber; it is here that we get acquainted with the history and importance of cucumber, its role in the household, its botanical description, varieties and biological requirements. The second chapter discusses the best known cultivation method: field production in full details. The presentation of the new varieties is especially important. The third chapter describes each phase of greenhouse forcing one by one and even presents the new method of hydroponic greenhouse production. Chapter four deals with hot-bed forcing; here too, as in the previous chapter, we can read about not only the cultural practices, but also the questions of profitability. This chapter (like the previous ones) pays great attention to the question of variety. Chapters five and six sum up the cultivation aspects of forcing in cold-beds and polyvinyl tents respectively, but only in a few words, as these production methods are not very wide-spread in Hungary so far. The seventh chapter deals with seed production. Regions suitable for seed production are spoken of separately, followed by questions of agrotechnics and variety maintenance. Unfortunately, in spite of their great importance this chapter only deals briefly with the techniques of seed production, although it would have been worth paying more attention to this question. Chapter eight discusses the protection of cucumber, its diseases and pests as well as the methods of control, while the last chapter describes the more important methods of preservation. The book is completed by literary references on two pages indicating that the authors have made considerable use of the literature available.

The book is undoubtedly a valuable work which gives a full picture of all aspects of cucumber production.

GY. MÁNDY

Maximization of Agricultural Production. Proceedings of the first Symposium of the Agricultural Society of India, 1968 (Edited by P. K. Sen) Calcutta 1969.



MAXIMIZATION
OF
AGRICULTURAL PRODUCTION

Edited by
P. K. SEN

The Agricultural Society of India organized the Symposium on the "Maximization of Agricultural Production" in order to be able to formulate a concrete line of approach in the urgent national campaign for an all out break through in Indian Agriculture. The main objective of the Symposium was to discuss threadbare this topical problem with particular emphasis on an integrated approach from different angles. The approach included problems of plant and soil factors under the aegis of human endeavour through the gamut of economics and extension.

The Symposium was held on September 23rd to 25th, 1968 at the College of Agriculture, Banaras Hindu University, with delegates from different parts of the country representing universities, agricultural colleges and research institutions, agro-industries and farmers.

The Symposium was inaugurated by Prof. A. C. Joshi, the then Vice-Chancellor of the University. The Presidential Address was given by Shri I. Chatterjee, the President of the Agricultural Society, whose very interesting speech ended as follows: "Let it not be forgotten that agriculture is a function of husbanding and harnessing the potentialities of all its components that make up its sum total. In their proper orientation, integration and judicious exploitation lies the key towards maximization of production."

The Symposium was distributed over six sessions viz., I. Genetic Control, II. Physiological Responses, III. Agronomic Aspects, IV. Water Management, V. Weed Control, VI. Role of Rural Extension. The book sums up on 225 pages the 31 lectures delivered to the six sessions.

13 lectures were delivered to the session on *Genetic Control* (I) dealing with crops such as maize, rice, barley and brown sarson.

Comparative performance of the hybrid and composite maize varieties and the factors responsible for maintaining the stability of the composite varieties have been studied by D. Sharma under All-India Co-ordinated Maize Improvement Programme. The author has reviewed pertinent literature and has indicated that on the basis of the knowledge of genetic parameters and population architecture, breeding for the composite varieties in maize is the most practical approach for India.

S. P. Banerjee et al. studied the adaptability of new rice selections in respect of different plant characters at three locations and under three fertility gradients. The most important point that emerged from the variance analysis was that in respect of grain yield, the magnitude of genotype × environment interaction was not so high as to disturb the order of ranking of varieties in any environment. Furthermore the data revealed that a large portion of phenotypic variance for each character was genetic.

Colchicin induced tetraploids of cultivated rice were tested by A. K. Richharia. The data showed that the tetraploid plants have greater vigour in respect of many plant characters. The results indicate the possibilities of building up the gene pool for future improvement through amphiploidy.

G. Bhowmik and K. Das dealt with the

effect of thermal neutron on certain quantitative characters in barley. It was found that while selecting plants for culm length, only grain weight, among other quantitative characters, showed increase in the mean value of the M_3 population over that of the control.

The induced mutations in gamma irradiated barley were studied by H. M. Shrivastava and K. Das. The data relating to the frequency of mutation in different doses of gamma rays show a linear relationship between dose and mutations. The spectrum of chlorophyll mutation in relation to sterility shows a somewhat random distribution of the various mutants.

8 lectures were delivered at the session on *Physiological Responses* (II). S. Kathju and M. N. Tewari investigated the effect of IAA on the growth and free amino acid contents of *Medicago sativa* seedlings. The seedlings treated with 10 ppmm IAA showed the presence of an unknown spot after 72 hours of germination. At this concentration growth of the seedling was also promoted.

R. S. Choudhri and A. N. Singh studying the effect of growth regulating substances on growth yield and oil content of certain oilseed crops has found that lighter doses of IAA, 2.4-D and MH with spray application significantly increased the growth, dry matter content and seed yield. The oil content of seeds generally remained unaffected by lighter treatments.

O. K. Garg and J. N. Singh dealing with the physiological changes in leaves related to sex pointed out that such differences were not only restricted in monoecious species like *Laganeria vulgaris*, *Zea mays* but were also observed for dioecious species like *Carica papaya*. The leaf samples were analysed for total carbohydrates, total nitrogen contents catalase and respiratory activities.

Three papers dealt with sorghum. The data of D. Kamalavalli et al. proved that soaking of seeds (*Sorghum vulgare* Pers.) for one hour in water caused significant early emergence during 36—48 hours after soaking. Longer than 4 hours soaking delayed the emergence, which was effectively reversed by Gibberelic acid (GA_3) proportionally to its concentration. GA_3 stimulated shoot length, mesocothyl length and resulted in increased dry weight of 4 day-old seedlings. G. Verma and D. Lal used the GA_3 for foliar application from the age of two weeks till anthesis. An interesting morphological phenomenon observed in treated CHS-1 sorghum plants was the emergence of new inflorescence in the axils of the top leaves. There was normal grain formation in these secondary heads. S. B. Lall and B. N. Phadnawis studied the hydrocyanic acid synthesis in sorghum. The results revealed that HCN synthesis is interrelated with photosynthesis and protein synthesis which was affected by nitrogen nutrition in this crop.

U. S. Gupta and P. Kaur examining the nodules on the roots of grain (*Cicer arietuum* L.) sown in a virgin land have found the size and numbers of nodules abnormal. The nodulated plants had a seed increase of about 30 per cent and 25 per cent in the number of pods per plant. It appears that a new virulent strain of Rhizobium is present in that soil.

S. K. Gupta carried out a pot experiment to study the effect of ammonium sulphate on the solubilization of iron, manganese and carbon dioxide in waterlogged rice soils. The data show that application of ammonium sulphate did not have any marked effect on the decomposition of soil organic matter as shown by the content of dissolved CO_2. In the presence of decomposable organic matter, considerably more Fe^{++}, but not Mn^{++}, came into the solution than in the presence of ammonium sulphate.

5 lectures were delivered to the session on *Agronomic Aspects* (III). Two of them dealt with the nitrogen fertilization of bajra (*Pennisetum typhoides* Pers.). Data of M. Singh and S. C. Varma show that 120 kg of nitrogen per hectare was effective for grain and straw yield in all of the high yielding hybrids. However, the positive response in straw yield was obtained even up to 200 kg N/ha. In the field experiment of S. K. Agarwal et al. the yield response of two hybrids (T-55, HB-1) to the graded doses of nitrogen was found to be quadratic and the

most economic doses were 80 kg and 120 kg nitrogen per hectare.

Two other papers dealt with some interesting aspects of maise hybrids. T. P. Singh et al. investigated how long maize hybrids and varieties of different silking dates take to mature as determined by kernel moisture, thousand grain weight and shelling percentage. It appears from the data that the crop takes 36 days from 75 per cent silking to reach the stage of maturity, irrespective of whether a variety belongs to an early or late maturity group. R. Singh and L. B. Chaudhari studied the relationship between certain agronomic characters of six hybrids and yield. Ear length, ear diameter, number of kernel rows and number of kernels per row had positive and significant correlations with the yield of the maize hybrids in both the seasons (kharif, rabi) of 1966—67. Growth attributes namely, plant height, ear height and number of leaves per plant did not show any significant correlation with yield.

V. G. Gawai et al. studied the effect of various cultural practices (chipping intervals, fertilization, soil moisture regime) on forage yield and protein content of grass pasture mixture (*Dactylis glomerata* L., *Bromus inermis* Leyse). The yield of forage increased most by nitrogen fertilization but the protein content was reduced with increase of N rate up to 112 kg/ha and slightly increased with 224 kg/ha. The short intervals of irrigation gave the highest forage yield but protein content decreased with the increased soil moisture.

4 lectures were delivered to the session on *Water Management* (IV). Water relations of rice were investigated by D. K. D. Gupta and P. K. Sen in a pot experiment with three seasons, nitrogen levels and varieties and four different watering treatments. Data show that rice varieties perform best with the differential supply of water at various stages of growth and development. There seems to exist a mutual inter-dependence between water and nitrogen in respect of growth and yield. Under limited nitrogen supply higher levels of watering show relatively better performance and under low watering increased nitrogen levels give relatively high yield. When both the water and nitrogen are high the crop is adversely affected. A. P. Bhattacharya summed up the current irrigation practices in the State of U.P. and gave suggestions how to improve them for the crops of wheat and rice.

Soil moisture studies in linseed crop were conducted by C. R. K. Prasher et al. The value of the consumptive use of the linseed crop (*Linum usitatissimum* L.) ranged between 16.64 cm to 25.42 cm. The rate of water use was maximum towards flowering and grain development stages. Irrigation efficiency ranged from 68.29 to 74.73 per cent.

H. N. Shahi investigated the suitability of tensiometers for scheduling irrigation in sandy loam soils. The instrument was found to be quite suitable for scheduling irrigation of field crops in sandy loam soils. The majority of the field crops, particularly wheat and maize need irrigation when the level of available soil moisture in the active root zone is about 50 per cent. Some crops like potato and high yielding dwarf varieties of wheat need irrigation still earlier (at 80—85 per cent).

4 lectures were delivered to the session on *Weed Control* (V) for such important crops as rice, cotton and wheat.

C. Thakur studied weed control in rice using chemicals as 2.4-D or MCPA alone and Stam F-34 alone or combined with MCPA as post-emergence application. S. K. Mukhopadhyay and S. P. Bhattacharya dealt with the chemical Tok E-25 for pre-emergence application. Due to costly weeding operations by mechanical and intercultural operations the control of weeds by the use of both types of chemicals (pre- or post-emergence application) has assumed great importance in rice cultivation. The results of both experiments were very significant, giving the best dose and time of application for both the monocot and dicot weeds in rice fields.

To control weeds in cotton S. N. Kaushik et al. carried out field experiments with different herbicides. Among the chemicals like Prometryn, Pataron, Simazin and

Dicryl, the first one was found the most effective (2 kg a.i./ha) in controlling weeds and increasing the seed cotton yield.

O. N. Mehrotra et al. dealt with the chemical control of weeds in Mexican dwarf wheat (Sonora 64). Application of a new pre-emergence herbicide BV 201 effectively controlled the majority of the broad leaved weeds and proved its superiority over 2.4-D formulations.

4 lectures were delivered to the session on the *Role of Rural Extension* (VI) which dealt with the important question that increasing agricultural production is not only a technical problem in India.

T. P. Singh pointed out that the first task of each extension worker should be to raise the level of aspirations of farmers. But there is still a need to classify all aspirations and find out which one effects the adaptation of improved agricultural practices most and to what extent.

V. Lakshminarayana analysed the socio-agro-economic changes in the village Goalpara. It seems that adaptation of improved practices in agriculture has been only marginal. This may be due to the weakness of the extension agency and lack of enterprise on the part of the villagers. The factor responsible for lack of enterprise is that most of the villages have Brahmin majority who also happen to be the land-owning class with higher level of literacy and education.

Another village in West Bengal was studied by P. K. Sen et al. Land and man-power in the village are disorganized. It is considered possible to change the situation and bring about economic prosperity to the village through a reoriented socio-economic approach accepting the village as one living system.

The last chapter has summarized the recommendation of the Plenary Session with a number of important tasks having practical bearing on the immediate problems of agricultural production in India.

The results of some research aspects presented by this book provides useful information for scientists working outside India too.

I. Simon Kiss

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 23 (1—2), pp. 261—264 (1974)

AUCTORES

Ahuja V. P.
Cummings Laboratory,
Indian Agricultural Research Institute,
New Delhi-12,
India

Ali S.
Department of Chemistry,
University of Allahabad,
Allahabad,
India

Antoni F.
SOTE Orvosi Vegytani Intézete,
1088 Budapest,
Puskin u. 9.
Hungary

Austin A.
Cummings Laboratory,
Indian Agricultural Research Institute,
New Delhi-12,
India

Basu N. C.
Jute Agricultural Research Institute,
Barrackpore (W. Bengal)
India

Berzsenyi-Janosits L.
8360 Keszthely,
Szendrey J. u. 1.
Hungary

Bocz E.
AE Növénytermesztési Tanszék,
4001 Debrecen,
Böszörményi út 138,
Hungary

Darwish A.
Faculty of Agricultural,
Assuit University,
Assuit,
UAR

De S. K.
Department of Chemistry,
University of Allahabad,
Allahabad,
India

Degenhardt W.
OPTON Feintechnik GmBH,
7082 Oberkochen,
Postfach 35/36,
G.F.R.

Dubey P. S.
Department of Botany,
University of Jodhpur,
Jodhpur,
India

Együd L. G.
Institute for Muscle Research,
P. O. Box 342.
Woods Hole, Mass. 02543,
USA

El-Ghayaty S. H.
Department of Plant Production,
Faculty of Agriculture,
Azhar University,
Azhar,
UAR

El-Kadi M.
Department of Plant Physiology,
Faculty of Agriculture,
Ain Shams University,
Ain Shams,
UAR

Esmail A. A.
Department of Food Technology,
Department of Food Control,
Assuit,
UAR

Farkas-Riedel L.
ELTE Alkalmazott Növénytani és
Szövetfejlődéstani Tanszék,
1088 Budapest,
Múzeum krt 4/a.
Hungary

AUCTORES

Fazekas S.
SOTE Biokémiai Intézete,
1088 Budapest,
Puskin u. 9,
Hungary

Fodor G.
Department of Chemistry,
West Virginia University,
Morgantown,
West Virginia 26506,
USA

Gangwar M. S.
Experiment Station,
U.P. Agricultural University,
Pantnagar (Distt. Nainital)
U. P. India

Gábos D.
Mezőgazdasági Technikum,
9300 Csorna,
Hungary

Gracza P.
KE Növénytani Tanszék,
1118 Budapest,
Ménesi út 44.
Hungary

Győri D.
AE Talajtani Tanszék,
8360 Keszthely,
Deák F. u. 16,
Hungary

Halmos M.
JATE Szerves Kémiai Intézet,
6720 Szeged,
Dóm tér 8.
Hungary

Hefni El-S.
Department of Agronomy,
Faculty of Agriculture,
Ain Shams University,
Cairo,
Egypt

Hegedűs Á.
Szőlészeti és Borászati Kutató Intézet,
1022 Budapest,
Herman Ottó út 15,
Hungary

Horn P.
Mezőgazdasági Főiskola,
7400 Kaposvár,
Hungary

Ibrahim A. N.
Faculty of Agriculture,
Al-Azhar University,
Cairo,
UAR

Kárpáti Z.
KE Növénytani Tanszék,
1118 Budapest,
Ménesi út 44.
Hungary

Kása I.
BME Kémiai Technológiai Tanszék,
1111 Budapest,
Műegyetem rkp. 3/9,
Hungary

Kecskés M.
MTA Talajtani és Agrokémiai
Kutató Intézete,
1022 Budapest,
Herman Ottó út 15.
Hungary

Kovács K.
JATE Szerves Kémiai Intézet,
6720 Szeged,
Dóm tér 8.
Hungary

Kurnik E.
Takarmánytermesztési Kutató Intézet,
7095 Iregszemcse,
Hungary

Lotfi A. Y.
Department of Food Technology,
Department of Food Control,
Assuit University,
Assuit,
UAR

Mall L. P.
Department of Botany,
University of Jodhpur,
Jodhpur,
India

Marmasse C.
Biophysical Laboratory,
Faculty of Sciences,
National University of Mexico,
U.N.A.M.
Mexico 20, D.F.

Mándy Gy.
AE Növénytani és Növényélettani Tanszék,
4001 Debrecen,
Böszörményi út 138.
Hungary

Mitra G. C.
Jute Agricultural Research Institute,
Barrackpore (W. Bengal),
India

Mukherji S.
Department of Botany,
University of Calcutta,
Calcutta-19,
India

Nakajima T.
The Department of Neurology,
Institute of Higher Nervous Activity,
Osaka University Medical School,
Fukushima-ku, Osaka
Japan

Nyéki J.
KE Növénynemesítéstani Tanszék,
1118 Budapest,
Ménesi út 44.
Hungary

Palit D.
Department of Chemistry,
University of Allahabad,
Allahabad,
India

Palkovics M.
AE Talajtani Tanszék,
8360 Keszthely,
Deák F. u. 16,
Hungary

Papp L.
Természettudományi Múzeum Állattára,
1088 Budapest,
Baross u. 13.
Hungary

Paul A. K.
Department of Botany,
University of Calcutta,
Calcutta-19,
India

Penke B.
JATE Szerves Kémiai Intézet,
6720 Szeged,
Dóm tér 8.
Hungary

Pozsár B. I.
Agrobotanikai Intézet,
2766 Tápiószele,
Hungary

Raafat A.
Department of Plant Physiology,
Faculty of Agriculture,
Ain Shams University,
Ain Shams,
UAR

Reporter M.
Charles F. Kettering Research Laboratory,
150 East South College Street,
Yellow Springs, Ohio 45387,
USA

Sáringer Gy.
Növényvédelmi Kutató Intézet
Laboratóriuma,
8360 Keszthely,
Felszabadulás u. 1/A,
Hungary

Sharma P. D.
Experiment Station,
U.P. Agricultural University,
Pantnagar (Distt. Nainital)
U.P. India

Simon-Kiss I.
Öntözési Kutató Intézet,
5540 Szarvas,
Hungary

Singh R. R.
Experiment Station,
U.P. Agricultural University,
Pantnagar (Distt. Nainital)
U.P. India

Singh T. A.
Experiment Station,
U.P. Agricultural University,
Pantnagar (Distt. Nainital)
U.P. India

Soó R.
ELTE Botanikus Kert,
1083 Budapest,
Illés u. 25.
Hungary

Surányi D.
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Kutató Állomása,
2700 Cegléd,
Hungary

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JATE Szerves Kémiai Intézet,
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Dóm tér 8.
Hungary

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SOTE I. sz. Kórbonctani Intézete,
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Üllői út 26.
Hungary

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Laboratory of the Institute for Muscle
Research at the Marine Biological
Laboratory,
Woods Hole, Massachusetts 02543,
USA

SZÉKESSY-HERMANN V.
SOTE Biokémiai Intézet,
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Puskin u. 9,
Hungary

TÓTH O.
AE Növénytermesztési Tanszék,
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Hungary

VETTER J.
ELTE Növényélettani Tanszék,
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Múzeum krt. 4/a,
Hungary

YAOI Y.
National Cancer Center Research Institute,
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Chuo-ku, Tokyo,
Japan

YOUSSEF K. E.
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ФИЗИОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ СОЛЕУСТОЙЧИВОСТИ PISUM SATIVUM L.
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Д. С. УПРЕТИ, М. Н. САРИН

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КАЧЕСТВЕННОЕ И КОЛИЧЕСТВЕННОЕ ИССЛЕДОВАНИЕ ПРИСУТСТВУЮЩИХ В СЕМЯДОЛЯХ ФОРМ АУКСИНА И ИХ ФИЗИОЛОГИЧЕСКОЕ ЗНАЧЕНИЕ

М. ВАРГА, И. ЛЕНАРТ

Обрезка и удаление семядольных листьев с черенков-гипокотилей разных проростков показали, что семядоли обладают сильной, стимулирующей укоренение индукцией, которую не может заменить верхушка побега. Следовательно, во всех случаях главный источник гормонов, индуцирующих корень, это — семядоли. Стимулирующий материал, вырабатываемый семядолями, является тождественным индолуксусной кислоте, т. к. экзогенная индолуксусная кислота в большой степени заменяет устраненные семядоли и т. к. 2,3,5, трийодбензолкислота, нанесенная под семядоли обезглавленных черенков-гипокотилей препятствует адвентивному формированию корня. Из семядольных листьев люпина изолировали 13—14 различных индольных соединений и большинство их было определено. В семядолях сухих семян находятся все известные формы индолуксусной кислоты в преформированном состоянии. В течение прорастания наблюдается синтез свободной индолуксусной кислоты, а начальная быстрая аккумуляция ауксина происходит путем реутилизации триптофана, освобождаемого во время гидролиза белков. Среди конъюгатов индолуксусной кислоты в семядолях находятся в значительном количестве индолацетиласпартат и индолацетил-β-Д-глюкоза и арабиноза, а как другие связанные формы ауксина в небольшом количестве индолуксусная кислота, связанная с макромолекулами. Уровень, связанных форм индолуксусной кислоты с начала прорастания быстро понижается, из чего можно заключить о их резервной роли. В семядолях, **in vivo**, осуществлялась конверсия триптофана-2-C^{14} → C^{14}-индолуксусной кислоты, и большая часть маркированного соединения аккумулировалась в зачатках корня.

ПОВРЕЖДЕНИЯ И УЩЕРБ, ВЫЗВАННЫЕ TANYMECUS DILATICOLLIS GYLL. НА ЛИСТЬЯХ И В УРОЖАЕ

А. КАЧО

В работе автор отчитывается о потерях в урожае, вызванных *Tanymecus dilaticollis* Gyll. путем повреждения листьев. Под изолятором листва кукурузы с 2—3 листьями повреждалась более чем двумя вредителями, приходящимися на одно растение, а достоверный ущерб в урожае вызван более чем 4 вредителями. В оранжерее 0.1—60 процентное повреждение листьев оказывает стимулирующее влияние на рост кукурузы с двумя листьями. Для 37 гибридов и одного сорта, использованных в полевых опытах, было характерно то, что погибшая листва хорошо воспроизводилась, но понижение урожая может быть устранено только в небольшой степени. Экономический порог — 20% ущерб листьев.

СУТОЧНЫЕ И СЕЗОННЫЕ ИЗМЕНЕНИЯ РАСТВОРИМЫХ УГЛЕВОДОВ У ANDROPOGON GAYANUS (ТРАВА СЕВЕРНАЯ ГАМБА)

А. А. АДЕГБОЛА, Е. БАЛОГ

Andropogon gayanus обычно встречается на естественных пастбищах Нигерии, и это привело на мысль изучить его питательную ценность, а также изменение физиологических факторов, влияющих на рост. Авторы изучали в различных частях растений количественные изменения растворимых углеводов и крахмала — главных источников запаса энергии в траве Гамба. Экспериментальные растения были выращены в Хозяйстве Университета Айф. Образцы брались регулярно от июня 1969 до июня 1970 г. Отделенные части растений анализировались по содержанию сахара и крахмала методом **Block** и **Pucher** ***et al.*** Установлено, что в различных частях растений всегда имелись сахароза, глюкоза и фруктоза в различном количестве. Иногда встречалось также небольшое количество мальтозы, ксилозы и арабинозы. Характер изменений подробно обсужден в опубликованной работе. Обсуждены также изменения в содержании крахмала в течение роста. В заключение можно сказать, что не было найдено прямой связи между продукцией углеводов в течение фотосинтеза и концентрацией свободных сахаров в растении. Литературные данные и наши результаты показывают на необходимость изучения в дальнейшем регуляционных систем, влияющих на питательность травы Гамба, и ее использование для получения силоса хорошего качества.

КОАГУЛЯЦИЯ ИНКУБИРОВАННЫХ ЯИЦ ВЫСОКОЧАСТОТНОЙ ОСЦИЛЛЯЦИЕЙ С ЦЕЛЬЮ ПАТОЛОГИЧЕСКИХ ИССЛЕДОВАНИЙ

Й. НАДЬ, А. ПАЛ

С помощью коагуляции, вызванной высокочастотной осцилляцией, исследовался метод фиксации клинических симптомов разложившихся яиц, который не деформирует признак болезни и не задерживает культуру патогенов. По нашим наблюдениям это условие обеспечивается следующими физическими свойствами. Гусиные яйца среднего размера после 8—10 дней инкубации могут быть коагулированы в среднем за 15 мин. в обмотке высокочастотного осциллятора. В случае куриных яиц среднего размера после 8—10 дней инкубации требовалось 12 мин. для коагуляции с помощью осциллятора меньшей мощности и подходящим диаметром обмотки. Согласно результатам нашего эксперимента, анодный ток осцилляторов показывает очень тесную корреляцию с удельным весом яиц. Результаты микробиологического исследования следующие. На средах картофель-б и пивном агаре грибы, принадлежащие к родам **Aspergillus**, **Penicilium** и **Mucor**, культивировались на образцах, полученных после коагуляции высокочастотной осцилляцией. На мясном агаре и среде **Klimert** культивировались колонии **E. coli**, **Streptococcus** и **Staphylococcus** после коагуляции.

СЕЗОННОЕ ИЗМЕНЕНИЕ СОДЕРЖАНИЯ К И Са У КСЕРОФИТНЫХ ВИДОВ ПОЧВЕННОГО ЛИШАЙНИКА И ИХ ПОЧВ

Е. КОВАЧ-ЛАНГ, К. ВЕРШЕГИ

На протяжении двух лет исследовалось сезонное изменение содержания К и Са у четырех ксерофитных видов лишайника *(Cladonia magyarica*, *Cladonia furcata*, *Cladonia convoluta* и *Parmelia pokornyi)* на песчанных дернах *Brometum tectorum secaletosum* и *Festucetum vaginatae.* Содержание Са в лишайниках оказалось гораздо более высоким, круговорот Са более быстрый, время обмена — гораздо короче, чем калия. Принимая во внимание фитомассу лишайников, количество Са в них и быстроту транспорта, можно определить, что их роль в транспорте Са у исследованных ценозов — более значительна, чем входящих в тот же ценоз трав. Роль лишайников ценоза является гораздо меньшей в транспорте К.

ГЕНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ КОМБИНАЦИЙ AEGILOPS × TRITICUM.

I. AE. TRIUNCIALIS × TRITICUM

К. К. ГОШАЛ, А. БЕЛЕА

Хотя геномное родство между *Aegilops* и *Triticum,* а также эволюция разных их видов уже обстоятельно разработаны, они не всегда ведут себя соответственно приписанным им геномным формулам. В последнее время в значительной мере переосмыслена филогенетическая близость этих двух родов. Наше исследование было частью разработанной программы, проведенной с целью чтобы собрать больше данных об их скрещиваемости, о плодовитости гибридов, а также об их цитологических и морфологических признаках. *Ae. triuncialis* по- разному скрещивается с тетраплоидным и гексаплоидным *Triticum.* Процент завязывания и проращивание гибридных семян были поразительно высокими в комбинации с гексаплоидным *Triticum.* Плодовитость гибридного поколения F_1 была не очень высокой. Цитологический анализ показал конъюгацию хромосом в мейозе у гибридов. Морфологически гибриды в большинстве случаев обладали промежуточными признаками. Повидимому геномная формула, приданная *Ae. triuncialis*, требует ревизии, для того чтобы объяснить высокое завязывание и прорастание гибридных семян, а также конъюгацию хромосом в мейозе, наблюдавшиеся в комбинациях *Ae. triuncialis* и тетраплоидных и гексаплоидных видов *Triticum.*

НЕМАТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ МАТЕРИАЛОВ, ИСПОЛЬЗОВАННЫХ ДЛЯ ПОКРЫТИЯ ГРИБНЫХ ГРЯД И ВОЗМОЖНОСТИ ЗАЩИТЫ

К. ФАРКАШ, И. КОРОНЦИ

Авторы собрали 6 видов покровного материала из 14 хозяйств Венгрии, занимающихся разведением грибов. На основе математической обработки выяснилось, что известняковый щебень и песок из шахт были незначительными источниками поражения. Из известнякового щебня был выделен 1 вид нематод, из парниковой почвы — 12, из торфа — 13, из среднесуглинистой полевой почвы — 24 вида нематод. Пропорция микопатологических видов составила в известняковом щебне 0,0%, в торфе — 1,14%, в парниковой почве — 2,98%, а в полевой почве — 8,28%. Обеззараживание покровных материалов формалином (4 литра/м^3) по эффективности не оказалось удовлетворительным (39,21% и 45,71%) ни в лабораторных, ни в заводских условиях. Эффективность специальных нематицидов при дозе 100 см^3/м^3 не оказалась тотальной ни в заводских, ни в лабораторных условиях. Их влияние на pH — незначительное. Несмотря на несовершенное нематицидное действие, они оказали благоприятное влияние на урожай. В заводском эксперименте по обеззараживанию покровных материалов — за исключением **Vapam** — все использованные изделия имели тотальное нематицидное влияние. С точки зрения урожая наилучшими оказались **Trapex, Nemagon, Fumason** и **Shell DD.**

ВЛИЯНИЕ БЕНЗИМИДАЗОЛА И ЕГО ПРОИЗВОДНЫХ НА ИНТЕНСИВНОСТЬ ФОТОСИНТЕТИЧЕСКОЙ ФИКСАЦИИ ДВУОКИСИ УГЛЕРОДА В ЛИСТЬЯХ ЛЮЦЕРНЫ И КУКУРУЗЫ

Л. ХОРВАТ, Б. И. ПОЖАР

Авторы показали значительное усиление интенсивности фотосинтетической фиксации двуокиси углерода под влиянием бензимидазола и его производных в таком раннем периоде обработки, когда уже можно проявить стимуляцию интенсивности белкового синтеза, хотя биологическую эффективность, повышающую уровень белков, пока еще нельзя показать. Надо подчеркнуть, что стимуляция, вызванная основной базой — больше, чем влияние производных. В отношении специфической активности стимуляция, вызванная бензимидазолом в листьях кукурузы, намного больше, чем в случае люцерны. Авторы пришли к выводу, что факторы резистенции типа бензимидазола (системных фунгицидов) стимулируют фотосинтетическую фиксацию двуокиси углерода, что является одним из благоприятных побочных действий этих соединений, совместно со стимуляцией белкового синтеза.

ВЛИЯНИЕ ДЕГРАНОЛА НА ФОРМИРОВАНИЕ УСТЬИЦ НА СЕМЯДОЛЬНОМ ЛИСТЕ СЕЯНЦЕВ

П. БАЛОГ, А. КЕРЕСТЕШ

Авторы исследовали влияние Дегранола в концентрациях 10^{-4} M—10^{-2} M на дифференцирующий эпидермис сеянцев лука с помощью светового микроскопа. Установлено, что в более концентрированных растворах понижается число устьиц на единицу площади, а материнские клетки устьиц часто становятся ненормально большими, и их ядра тоже увеличиваются. Форма окружающих клеток эпидермиса в значительной мере не отличается от контроля. Авторы сопоставляют свои результаты с результатами, полученными от действия колхицина на эпидермис лука с одной стороны, и Дегранола — на тканевые культуры животных и людей — с другой.

ЦИТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЛИЯНИЯ НЕСКОЛЬКИХ МУТАГЕННЫХ АГЕНТОВ НА ГОРОХ (PISUM SATIVUM L.)

ВО ХУНГ, Б. НАДЬ-ПОРПАЦИ

Исследовалось цитологическое влияние нескольких мутагенных агентов на митоз и мейоз гороха. Сорта, использованные в эксперименте, по-разному реагировали на различные обработки мутагеном. При обработке 0.05% N-нитрозо-метилкарбамидом сорта **Gloria di Quimper** были найдены очень длинные, вытянутые, деформированные клетки. В интерфазе наблюдались многоядерные клетки. Под влиянием обработки 0.1 процентным этилметансульфонатом процесс деления проходил нормально. Под влиянием обработки 0.3 процентным этилметансульфонатом у трех сортов были найдены 2—3-ядерные клетки. Рентгеновское облучение при дозе 5, 10 кр не вызвало нарушения деления, но при дозе в 15 кр наблюдались хромосомные и хроматидные аберрации. Обработка гамма-лучами в 5 кр и 0.1% этилметансульфонатом в метафазе митоза вызвало сцепление хромосом в клубки. В интерфазе встречались 2—3-ядерные клетки. Гамма-облучение при дозах в 5, 10, 15, 20 и 25 кр сильно задержало деление у сортов **Mansfelder Grüne** и **Zenith**. Сорта **Gloria di Quimper** и **Bärdi** чувствительно реагировали на гамма-облучение, при всех дозах наблюдалось нарушение деления. В метафазе митоза наблюдались хромосомные и хроматидные разрывы, в анафазе — хромосомные и хроматидные мосты. Нарушенное деление наблюдалось и в мейозе. В поколении M_2 аберрации продолжали появляться в митозе и в мейозе, но частота их по сравнению с поколением M_1 сильно снизилась.

БЫСТРОЕ ОПРЕДЕЛЕНИЕ СОДЕРЖАНИЯ КАЗЕИНА В ПРОИЗВОДСТВЕННОМ НАТУРАЛЬНОМ МОЛОКЕ, С ОСОБЫМ ВНИМАНИЕМ НА ПАТОЛОГИЧЕСКОЕ МОЛОКО

А. ВАГНЕР, И. МЕРЕНИ, М. ДОБОШ КОВАЧ

Авторы выработали непосредственный и быстрый метод для определения содержания казеина в молоке. Принцип метода заключается в том, что казеин исследуемого молока обратимо осаждается добавлением 3М водного раствора сульфата аммония, потом осадок вновь растворяется в 0,85% растворе кухонной соли и восстанавливают исходный объем молока. Затем с помощью реакции окрашивания белков (амидочерный 10 Б) фотометрически измеряется содержание казеина полученного раствора. Этим методом исследовалось молоко различных четвертей вымени, используя отрицательную и положительную реакцию Schalm, и установлено, что по отношению к общему белку содержание казеина составляет 77—80% или выше 80% у животных со здоровым выменем. Состав молока, содержащего меньше 77% казеина, является ненормальным. Этот метод рекомендуется при приеме молока по содержанию белка и для анализа на молочном заводе.

АКТИВНОСТЬ ПЕКТИНЕСТЕРАЗЫ У ПЯТИ СОРТОВ ИРАКСКОЙ ЯБЛОНИ В РАЗЛИЧНЫХ СТАДИЯХ ЗРЕЛОСТИ

Х. А. АЛ-ДЖАЗИМ, С. Х. АЛИ

Наблюдались изменения в активности пектинэстеразы (ПЭ) у пяти сортов иракской яблони в различных стадиях зрелости. Активность энзима была определена с помощью титрования карбоксиловой группы, полученной путем гидролиза пектина с разведенным NaOH. Полученные результаты показывают, что в этих сортах активность ПЭ увеличивалась в период прохождения созревания. Максимальная активность энзима наблюдалась в то время, когда фрукты были вполне зрелыми и готовыми к коммерческому сбору. За увеличением активности энзимов затем следовало общее понижение, когда фрукты созрели на дереве. Данные указывают и на качественное различие в поведении этих сортов.

ЕСТЕСТВЕННАЯ ПАРТЕНОКАРПИЯ В СОРТАХ ГРУШИ

Й. НИЕКИ

Естественная партенокарпия 37 сортов груши изучалась в 1968, 1969 и 1970 гг., каждый год у разного числа сортов. На основании результатов установлено, что партенокарпические плоды являются в первую очередь генетически детерминированной характеристикой сорта. Однако, на ее проявление влияют экологические факторы. Отношение завязавшихся партенокарпических плодов к числу созревших плодов может меняться из года в год. Согласно нашим исследованиям, высокие температуры (20—25°C), преобладающие во время завязывания плодов, положительно влияют на проявление партенокарпии. Согласно величины естественной партенокарпии сорта могут быть разделены на шесть групп. Зрелые плоды — либо полностью бессемянные или содержащие мешки, состоящие только из семенных оболочек, были получены у 3,4 процента обследованных цветков. В исследованных сортах апомиксис не был замечен.

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JÓZSEF SCHANDL

(1885—1973)

József Schandl, Kossuth Prize winner academician was followed on his last way on 17 July 1973 by the deep feelings of his friends, admirers, pupils and of numerous representatives of the scientific life of Hungary. Hungarian agriculture has again lost an outstanding personality. It is not very likely that one can find agricultural experts in Hungary who did not know — if not personally, at least through his books — Prof. Schandl, the recognized leader of Hungarian animal husbandry for long decades. With him an excellent teacher has gone who, with the power of his personality and richness of his knowledge and experiences, held his audience spell-bound not only in the course of his nearly forty years' carrier as a teacher, but often in the seventh and eighth decade of his life, too.

József Schandl was born on 27 April 1885 at Bakonybél, a little village in Transdanubia. Having completed his secondary school and college studies, he obtained a diploma at the Agricultural Academy of Magyaróvár in 1906, then in 1909 graduated at the Veterinary College. After his military service in Vienna he returned to the Veterinary College, where he worked under the guidance of Oszkár Wellmann academician and took part in series of experiments of international importance on metabolism. He was appointed agricultural college demonstrator in 1911, and assistant professor in 1912. After a study tour abroad, during which he got acquainted with animal husbandry in Germany, Austria, Holland, Denmark and Sweden, he was appointed head of the department of animal production at the Agricultural College of Kolozsvár in 1913. After his military service in World War I he taught from

1919 as an assistant professor at the Agricultural College of Magyaróvár, then from 1924 as a full professor at the Faculty of Economics and later at the Faculty of Agriculture of the József Nádor University of Technical and Economic Sciences in Budapest, and finally, as head of the department of animal production at the Faculty of Agriculture of the University of Agricultural Sciences, until 1948. Parallel with his activity as a university professor he performed the management of the National Institute for Wool Qualification; by developing this institution and organizing the herd-book keeping of sheep in Hungary he rendered great services.

He took an active part in the work of various state and social organizations. He was member of the National Council of Economic Education, the National Council of Veterinary Hygiene, of the Council of Agricultural Experimentation, the National Council of Natural Sciences, the Agricultural Scientific Council, and for long years president of the Committee of Animal Production of the Hungarian Academy of Sciences. From 1948 he worked at the Research Institute of Animal Production and made investigations mainly in the subject of sheep breeding; then from 1951 took over the management of the Institute which he continued up to his retirement in 1960. Especially at this last post of his he shaped the course of research work in more than one field of animal production for a decade. In 1952 he was conferred the title of Doctor of Agricultural Sciences by the Scientific Qualification Committee. In 1953 he was elected corresponding, and in 1960 ordinary member by the general assembly of the Hungarian Academy of Sciences.

Although the consequences of the two World Wars cast their shadow on the scientific and educational carrier of József Schandl, he was active participant of the revolutionary transformation of Hungarian agriculture, when, in spite of his advenced age, he became a pioneer of livestock-breeding methods the importance and perspectivic character of which was not always recognized even by the younger expert generations. It was sheep breeding and wool production that he always regarded as his special line, in which not only important works were published by him in the Hungarian and foreign literature, but many doctor's dissertations written under his guidance helped to clarify unsettled questions. The breeding of the Hungarian comb-wool merino is one of the results of his significant scientific work. He performed work by improving the milking capacity of the merino stock, and introducing the national registration and milk recording of sheep. His investigations were always aimed at clarifying practical questions. In sheep breeding he established a school and educated numerous outstanding experts for Hungary.

Schandl's untiring, conscientious and devoted activity in writing textbooks and technical books beside his work as a teacher and institute director, and other manifold public responsibilitie deserve particular attention. His text-books, in addition to making the education of university and college students more perfect and more efficient, also served as a guide for the practical experts for decades.

For his outstanding activity József Schandl was repeatedly given state, social and scientific decorations. He received the Order of the Red Banner in 1954, the Kossuth Prize in the same year, then in 1960 the Order of Labour; he was awarded an honorary doctorate at the University of Agricultural Sciences, Gödöllő, and at the Humboldt University, Berlin.

With Schandl's death a life rich in results ended. His memory, teaching and example will, however, survive and remain with us.

A. HORN

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN PISUM SATIVUM L.

I. GERMINATION AND SEEDLING GROWTH

By

D. C. Uprety, M. N. Sarin

INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

The interactive effect of soil salinity and growth regulators, on the germination, seedling survival and seedling growth of two varieties of *Pisum sativum* L. was studied. It was observed that soil salinity significantly depressed germination in the two varieties of peas. This deleterious effect on germination was due to (*a*) inhibition of sprouting of seeds, (*b*) slower velocity of germination and (*c*) mortality of seedling. An application of Phosfon-D to the soil ameliorated the adverse effect of soil salinity on germination not only by enhancing the sprouting of seeds but also by significantly reducing the mortality rate. The improvement in seedling growth as a result of the Phosfon-D treatment was due to its increasing the dry weight of the seedlings. GA_3 application improved the elongation of the plumule and the radicle but could not ameliorate the deleterious effect of soil salinity on germination, seedling survival, rate of germination and dry weight of seedlings.

Introduction

The stress due to the excessive accumulation of salts in the soil is experienced by most of the crop plants, as saline soils are widely distributed in arable lands of India. Germination has invariably been reported to be one of the most susceptible phases to salt injury and the adverse effect soil salinity had on it was said to be due to the osmotic inhibition of water intake by the imbibing seeds or by the accumulation of toxic quantities of constituent ions of saline soil or otherwise by a deranged metabolism (BERNSTEIN—HAYWARD 1958). The authors suggesting the above hypothesis drew their conclusion either from the studies on isoosmotic solution or the accumulation of specific ions or salt induced alterations in any phase of metabolism. These techniques are helpful in the elucidation of the gross salt damage but do not provide conclusive evidence for the precise effect, because there is no possibility of reconfirming the responses by reversing the salt stress. Such a reversal of salt damage will obviously afford a better means to check the observed responses. The reversal methodology will have an added advantage, as it may provide the basis for developing techniques which could be used for improving the seed germination and seedling growth.

In the present study, therefore, an attempt was made to understand the effect of soil salinity on germination, seedling survival and seedling growth of two varieties of *Pisum sativum* L. Growth regulators such as Gibberellic acid (GA_3) and Phosfon-D were tested for the reversal of the salt effect. *Pisum sativum* L. was selected for this study because this crop has been rated as sensitive to salt injury (Bernstein—Hayward 1958) and also because it is an important legume grown in those parts of this country which have large areas of salt affected land.

Material and method

Seeds of two varieties of *Pisum sativum* L., i.e. 'Rimpus' and 'Vares' were sown in petridishes containing (*a*) unsalinized soil (Electrical Conductivity (E.C.) of the saturation extract of the soil was 3.5 mmhos./cm at 25°C), (*b*) soil artificially salinized by the addition of an equal mixture of sodium chloride and calcium chloride to raise the E.C. of the saturation extract of the soil to 8.0 mmhos/cm at 25°C (salinized soil), (*c*) salinized soil supplied with 16 mg of 2,4-dichlorobenzyle tributyl phosfonium chloride (Phosfon-D) per 300 g of soil, (*d*) salinized soil supplied with 12 mg of gibberellic acid (GA_3) per 300 g of soil and (*e*) salinized soil supplied with a mixture of GA_3 and Phosfon-D (similar concentration). Various concentrations of these growth regulators were tested and it was found that with the above-mentioned doses these chemicals showed their maximum effect on germination (Uprety 1971).

Fifteen seeds were sown in each petridish and these were kept in a germinating chamber at 27° ($\pm$2°C). Ten petridishes were used for each treatment combination.

Seeds showing about a millimeter of radicle growth were ascribed as 'germinated' and a record of such germinated seeds was kept at 4 day intervals from 4 to 20 days after sowing. The number of seedlings that survived amongst the germinated seeds on the 20th day after sowing were also recorded. The rate of germination was calculated by Kotowski's method (1926). Observations were also recorded on the length of the radical and plumule and the dry weight of the seedling 96, 120 and 144 hours after sowing. All the data were analysed statistically by analysis of variance.

Results

The results of germination, coefficient of velocity of germination, seedling survival, length of plumule, length of radicle and dry weight of seedling are shown in Tables 1, 2, 3, 4, 5 and 6 respectively.

Soil salinity significantly depressed the germination of seeds at all the stages investigated in both the varieties. Germination reached about maximum 8 days after sowing in unsalinized soil whereas it was continued up to 16 and 12 days under salinized soil in var. 'Rimpus' and 'Vares', respectively. Seeds sown in Phosfon-D-treated salinized soil, showed a significant enhancement in their germination percentage as compared to those in untreated salinized soil, but this response was observed only 12 days after sowing in var. 'Rimpus' and 8 days after sowing in var. 'Vares'. An application of GA_3 and a combined treatment of GA_3 and Phosfon-D to the saline soil increased the germination of seeds at the initial stages (4 days after sowing), whereas a marked depression was observed for the rest of the period in GA_3-treated salinized soil. There was no significant effect of the combined application of GA_3 and Phosfon-D at later stages (Table 1).

Table 1

Interactive effect of soil salinity and growth regulators on the germination of seeds
(Germination percentage)

Variety	Days after sowing	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GA_3-treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	4	35	12	13	30	20	4.8
	8	72	35	35	40	40	4.4
	12	73	60	67	50	62	3.7
	16	73	66	72	58	66	3.8
	20	73	66	72	58	66	3.8
VARES	4	45	27	27	36	34	6.1
	8	82	42	57	46	50	7.3
	12	84	65	73	55	62	3.7
	16	84	65	73	59	62	3.8
	20	84	65	73	59	62	3.8

Table 2

Interactive effect of soil salinity and growth regulators on the coefficient of velocity of germination

Variety	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GaA-treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	6.0	4.1	4.9	3.6	4.1	1.2
VARES	6.9	4.0	4.5	3.6	4.6	1.5

Table 3

Interactive effect of soil salinity and growth regulators on the survival of seedlings
(Percentage of survived seedlings 20 days after sowing)

Variety	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GA_3-treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	93	85	92	77	82	5.8
VARES	96	75	89	75	81	6.6

Table 4

Interactive effect of soil salinity and growth regulators on the length of plumule
(Cms)

Variety	Hours after sowing	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GA_3 treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	96	0.63	0.24	0.15	0.83	0.60	0.25
	120	2.84	1.90	1.50	3.30	3.12	0.53
	144	5.82	4.20	2.92	5.44	5.42	1.22
VARES	96	0.96	0.73	0.58	1.42	0.79	0.20
	120	3.50	2.16	1.64	4.70	4.32	0.84
	144	7.40	4.72	3.50	7.00	7.94	1.86

Table 5

Interactive effect of soil salinity and growth regulators on the length of radicle
(Cms)

Variety	Hours after sowing	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GA_3 treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	96	3.68	2.42	1.58	3.54	3.34	0.16
	120	5.48	3.76	2.92	5.70	4.00	1.13
	144	8.40	6.54	4.64	8.42	7.08	1.03
VARES	96	5.02	2.80	2.14	4.14	2.90	0.31
	120	8.70	6.44	4.14	7.12	5.50	1.21
	144	10.94	7.76	5.66	10.34	8.30	1.17

Table 6

Interactive effect of soil salinity and growth regulators on the dry weight of seedling
(g)

Variety	Hours after sowing	Unsalinized soil	Salinized soil	Phosfon-D-treated salinized soil	GA_3 treated salinized soil	GA_3 + Phosfon-D-treated salinized soil	C.D. at 5% P
RIMPUS	96	0.097	0.071	0.096	0.065	0.087	0.008
	120	0.115	0.088	0.100	0.076	0.098	0.008
	144	0.130	0.094	0.108	0.087	0.103	0.013
VARES	96	0.090	0.074	0.076	0.056	0.079	0.013
	120	0.101	0.078	0.087	0.067	0.079	0.011
	144	0.112	0.082	0.101	0.075	0.095	0.010

In order to understand the influence of soil salinity on the rate of germination, the coefficient of the velocity of germination was calculated. It was observed that soil salinity brought about a marked reduction in the rate of germination in both the varieties. The application of Phosfon-D alone as well as in a combination with GA_3 did not bring about any significant improvement in the coefficient of the velocity of germination under saline conditions. However, the application of GA_3 to salinized soil further retarded the velocity of germination (Table 2).

Soil salinity not only reduced germination but also brought a significant reduction in the percentage of the survival of seedlings. An application of Phosfon-D to salinized soil increased the number of surviving seedlings, so much so, that they were significantly more than those grown in untreated salinized soil or soil treated with other growth regulators. An application of GA_3 had a further adverse effect on the survival of seedlings in var. 'Rimpus' but no conspicuous effect of this treatment was observed in var. 'Vares'. There was no significant change in the survival of seedlings due to the combined application of GA_3 and Phosfon-D in salinized soil (Table 3).

In addition to germination, the subsequent growth of the seedlings, as measured by their elongation and dry weight, was also adversely affected by soil salinity. This deleterious effect was manifest at all the stages investigated. An application of Phosfon-D to salinized soil reduced the length of the radicle more than the GA_3 or GA_3 and Phosfon-D treatments reduced seedling growth. These two latter treatments, however, markedly increased the radicle growth. The influence of soil salinity and growth-regulator treatments on plumule length was more or less similar to that on radicle growth (Table 4 and 5).

The dry weight of seedlings, grown in Phosfon-D-treated salinized soil, was significantly higher than that of those grown in untreated, GA_3-treated and GA_3- and Phosfon-D-treated salinized soil measured 96 and 144 hours after sowing in var. 'Rimpus' and 144 hours after sowing in var. 'Vares'. An application of GA_3 brought about a marked reduction in the dry weight of seedlings, whereas the combined treatment of GA_3 and Phosfon-D increased the dry weight of seedlings at 96 and 120 hours after sowing in var. 'Rimpus' and 144 hours in var. 'Vares' (Table 6).

The deleterious effect of soil salinity on germination has been observed both as delay in germination as well as reduction in the population of germinated seeds in various species of cereals and legumes (AYERS *et al.* 1952, GEORGE—WILLIAMS 1964, DONOVAN—DAY 1969). The present finding with the pea not only confirmed these observations but demonstrated that the deleterious effect of soil salinity on final germination was mediated by (*a*) inhibition of sprouting of seeds (*b*) lower velocity of germination and (*c*) mortality of seedlings.

Salt damage has been reported to be manifest not only in the germination phase but also during the subsequent growth of seedlings (AYERS *et al.* 1952, PEARSON *et al.* 1966). In the present study a decrease in early seedling growth was also observed as indicated by the lower elongation of the plumule and radicle and the reduced dry weight of the seedling.

The amelioration of salt injury to germination was achieved by an application of GA_3 by KAHN *et al.* (1957) in lettuce seeds and CHAUDHRI—WIEBE (1964) in wheat seeds. ODEGABARO—SMITH (1969) succeeded in improving the germination of *Lactuca sativa* seeds by the application of kinetin. In the present study with peas it was observed that only an application of Phosfon-D to saline soil appreciably improved the germination of seeds while GA_3 and a combination of GA_3 and Phosfon-D, although initially stimulated the germination it could not finally improve the same. Further, though the Phosfon-D treatment could not improve the elongation of the plumule and radicle as it is a growth retardant (CATHEY 1964), it ameliorated the adverse effect of soil salinity on dry-matter production. The increase in the elongation growth of the seedling due to GA_3 is a usual response of gibberellic acid (STUART—CATHEY 1964) but it could not enhance the dry-matter production.

Acknowledgement

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QUALITATIVE AND QUANTITATIVE STUDY AND BIOLOGICAL IMPORTANCE OF AUXINS OCCURRING IN COTYLEDONS

By

M. Varga, I. Lénárt

DEPARTMENT OF BOTANY, JÓZSEF ATTILA UNIVERSITY, SZEGED

By mutilating and removing the cotyledons of hypocotyle cuttings from various seedlings it has been proved that the cotyledons induce an intensive hormone activity stimulating the root formation which cannot be replaced by the activity of the shoot apex. Thus the main hormone source initiating the root formation is in every case the cotyledon. Since the exogenous IAA replaces to a great extent the removed cotyledons, and because the TIBA ring smeared below the cotyledons of decapitated hypocotyle cuttings prevents the formation of adventive roots, the stimulant originating from the cotyledons is identical with IAA. From cotyledons of lupine 13—14 different indole compounds were isolated and most of them identified. In the cotyledons of dry seeds all known forms of IAA were present in a preformed state. In the course of germination the free IAA shows an induced synthesis, and the initial rapid growth of auxin takes place by the reutilization of tryptophan released with the hydrolysis of proteins. Of the IAA conjugates considerable amounts of indole-acetyl-aspartate and indoleacetyl-β-D-glucose and arabinose, and, as another form of auxin, also a low amount of IAA bound to macromolecules can be found in the cotyledons. The level of the bound IAA forms rapidly decrease from the beginning of the germination which suggests their playing the role of some kind of reserves. In the cotyledons the conversion of tryptophan-2-^{14}C → ^{14}C-IAA has *in vivo* been realized and a large part of labelling has accumulated in the root initials.

Introduction

It is a well-known fact that a locally higher auxin concentration in various parts of the plants results in root formation, and that in tissue cultures too, an auxin surplus compared to the other hormones favours the initiation of roots. This rule plays an important role in the differentiation of organs, in the phenomena of polarity and restitution.

It was in the course of growth-physiological investigations made with various hypocotyle cuttings that the question arose: where the auxin-inducing root formation in the hypocotyles of young seedlings originates from. Hormone sources may be, namely, the known sites of IAA synthesis, the apical bud and the primary leaves, and — according to our observations — the cotyledons too. We therefore set the aim of performing detailed studies with a number of plants on the importance of cotyledons in root formation, determining the amounts and changes of level of IAA forms found in the cotyledons, further, of attempting to interpret the biological importance of these auxin forms.

Material and method

The experiments were performed with seedlings of cucumber (*Cucumis sativus* L. "Rajnai"), pumpkin (*Cucurbita pepo* L. "Korai fürtös") and lupine (*Lupinus albus* L. "Gyulatanyai édes").

Rooting of cuttings. The seedlings were raised in a growth chamber, in perlite containing 70 per cent water, at a temperature of 24 °C and light intensity of 8000 lux. The isolated shoots of five-days-old seedlings were cut to a length of 3.5 cm and fixed in a wire net covering glass vessels to develop roots in Hoagland solution containing 1 per cent saccharose. Rooting took six days, while the solution was renewed in the vessels every two days. The extent of root formation was determined by measuring the number and mass of roots formed per hypocotyle. Each treatment was performed with 10 cuttings in four replications.

Extraction and chromatography of indole compounds. The methanol extract made of the tissue homogenizate was purified by shaking it with petroleum ether, concentrated by evaporation in vacuum, then chromatographed on silicagel- G layer with a solvent of chloroform-ethylacetate-formic acid (5 : 4 : 1) and isopropanole-7% ammonia-water (8 : 1 : 1) (VARGA—BITÓ 1968).

The indole compounds were identified on the basis of the R_f values compared to those of the synthetic compounds, the colour obtained with Ehrlich's reagent and the UV fluorescence and UV absorption spectra.

Quantitative determination of free IAA and IAA conjugates. The corresponding chromatogram spots were scraped off the plate, eluated with methanol, and the amount of material found in the eluate measured with a Spektromom 202 photometer at 280 nm (FLETCHER—ZALIK 1964).

IAA bound to macromolecules was measured with the modified method of GALSTON *et al.* (1964). From the homogenized tissue all free IAA and IAA conjugates were removed by repeated extraction, then the tissue pulp was suspended in 2 N NaOH and hydrolysed at 60° C for 3 hours. After being centrifuged the hydrolyzate was acidified with HCl to pH 3, shaken three times with peroxide-free ethylether, and the collected etheric fraction — which contained the IAA released from the macromolecules — was evaporated to dryness. The material taken up with methanol was chromatographed in the above described way, and the IAA concentration measured by spectrophotometry.

In the isotope studies carboxyl-^{14}C-IAA (Szeged University, Department of Radiochemistry) and tryptophan-2-^{14}C (Central Research Institute of Chemistry) were used. The specific activity of the labelled IAA was 3.65 mCi/mM, that of the tryptophan 6.5 mCi/mM. The autoradiograms were prepared on a FORTE High Speed X-Ray film with an exposition time of 4 days.

The protein content was measured by a turbidimetric method, using serum albumine as a standard, according to BAGI—FARKAS (1967).

Each chemical analysis was made in three replications, with two parallels.

Results

It can be established for all three experimental plants that the extent of root formation — expressed either by the number or by the mass of the roots — is mostly proportionate to the surface of the cotyledon (Table 1). Root formation is thus initiated by a hormonal induction from the cotyledons, therefore, their mutilation considerably reduces, and their removal almost completely prevents the formation of adventitious roots (Fig. 1).

In all cases the extent of root formation is the highest when, besides the shoot apex, the cotyledons too are present. Essentially fewer roots are formed when only the leafy apex is left untouched than when the cotyledons are present with the apex removed (Figs. 2 and 3). Thus, in the adventitious root formation of young shoot cuttings the cotyledon is a more important — one

Table 1

Dependence of hypocotyl- root formation on the presence and surface of cotyledons (6th day)

Cotyledon surface, %	Cucumber		Lupine		Pumpkin	
	root number	root weight, mg	root number	root weight, mg	root number	root weight, mg
100.0	20.1	48.1	14.4	98.0	17.2	82.0
75.0	17.3	35.2	11.8	71.2	14.7	63.2
50.0	10.5	20.7	9.6	52.4	10.1	31.4
25.0	7.6	5.5	6.5	23.2	6.3	12.2
12.5	3.2	2.0	3.2	8.1	2.9	3.9
0.0	1.5	0.4	2.1	3.0	2.0	0.8

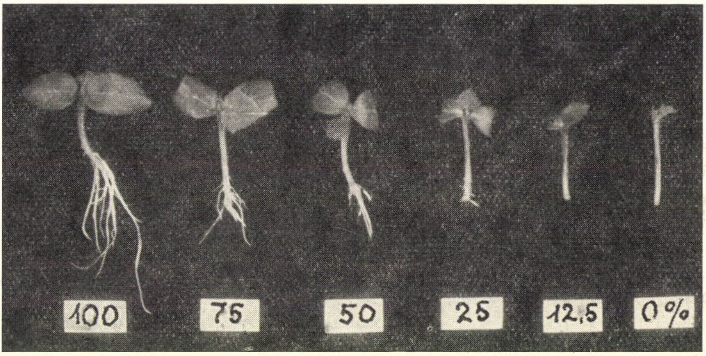

Fig. 1. Adventitious root formation of cucumber-hypocotyl cuttings in the case of different cotyledon surfaces (%)

might as well say the main — source of auxin, while the apex is of much lower importance; the quantitative ratio of the hormone originating from them is about 3 : 7 in cucumber and pumpkin and 2.4 : 4 in lupine, in favour of the cotyledon. So in the case of young seedlings the apex plays but a subordinate role in supplying hormone translocating to the base.

Exogenous IAA (1 and 10 ppm) added to the rooting medium can replace — though only to some extent — the stimulus originating from the cotyledons; it is, however, able to replace completely the action of the removed shoot apex (Table 2). This suggests again that the hormone induction originating from the cotyledons is more important, and is at the same time an indirect evidence of the root-forming factor transported from the cotyledons being IAA.

The 2,3,5-triiodobenzoic acid (TIBA) ring smeared below the cotyledons of decapitated hypocotyl cuttings inhibits root formation almost completely (Fig. 4). Since TIBA is a specific blocking agent of the basipetally polar trans-

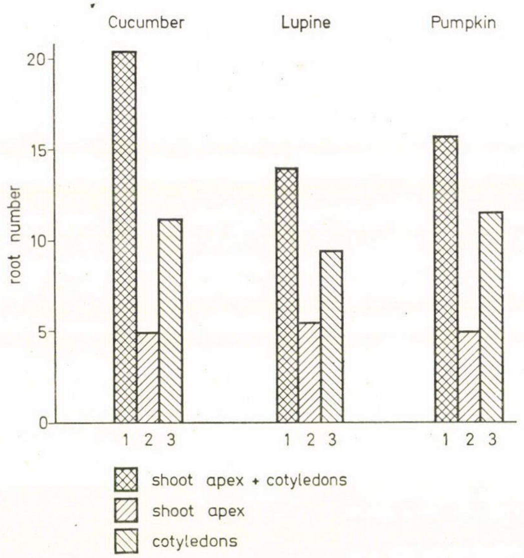

Fig. 2. Effect of the presence and removal of the apical bud on the number of adventitious roots formed on the hypocotyl. (1 = both the apical bud and the cotyledons are present, 2 = only the apical bud is present; 3 = only the cotyledons are present)

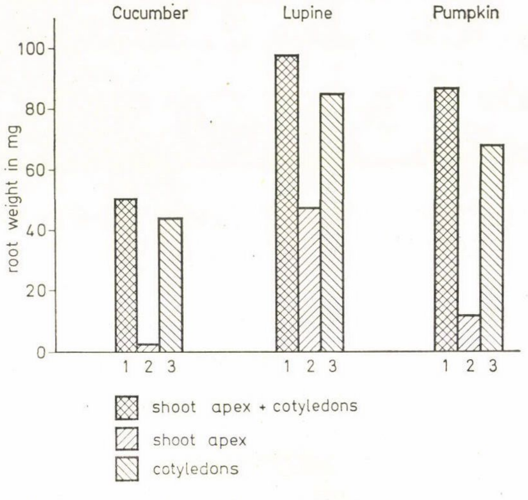

Fig. 3. Dependence of the mass of adventitious roots on the presence or removal of the apical bud. (1 = both the apical bud and the cotyledons are present; 2 = only the apical bud is present; 3 = only the cotyledons are present)

port of IAA (KUSE 1953, HAY 1956, NIEDERGANG-KAMIEN—SKOOG 1956, etc.), the hormone originating from the cotyledons and inducing root formation is really identical with IAA.

The above results agree in many respects with those obtained by KATSUMI *et al.* (1969) and AUNG (1972) who also pointed out the young coty-

Table 2

Root formation of hypocotyls in the presence of IAA, with and without cotyledons or shoot apex (6th day)

Hypocotyl cutting	Cucumber		Lupine		Pumpkin	
	number of roots		number of roots		number of roots	
	control	IAA 1 ppm	control	IAA 10 ppm	control	IAA 10 ppm
Shoot apex and cotyledons are present	19.7	24.5	13.9	22.0	15.4	23.1
Shoot apex present, cotyledons removed	5.0	9.0	5.9	7.1	5.8	7.2
Cotyledons present, shoot apex removed	13.7	25.2	9.8	21.1	10.3	21.6

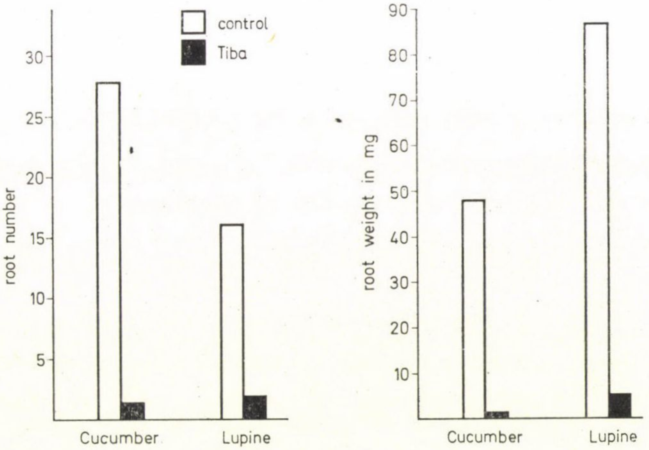

Fig. 4. Effect of TIBA ring smeared below the cotyledons on the root formation of cucumber and lupine hypocotyls. (1.5 per cent TIBA in lanolin paste)

ledons to be the primary places of the root-promoting substances which may in the first place be indole-auxins.

The root-initiating role of IAA transported from the cotyledons is proved by the autoradiograms, seen in Fig. 5, too. According to the evidence of these autoradiograms the radioactivity of carboxyl-^{14}C-IAA injected into the cotyledons accumulates in a great measure in the root initials of hypocotyles, and appears in the developed adventitious roots too. According to our investigations, some 75—80 per cent of the labelling found in the root initials belongs invariably to IAA, and only about 20—25 per cent to other compounds in which the $^{14}CO_2$ released by decarboxylation during the metabolism of IAA has become incorporated.

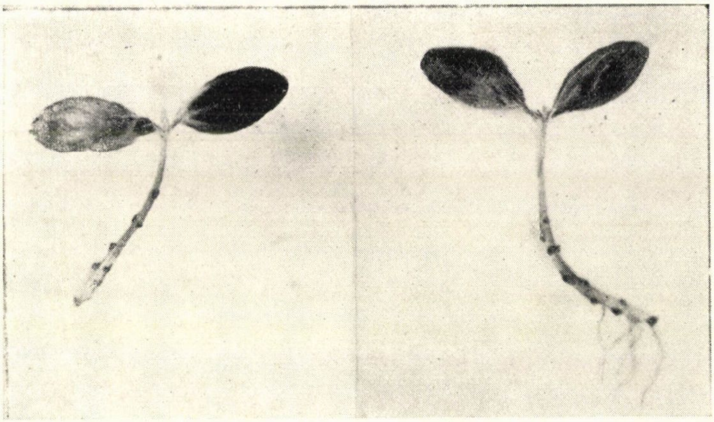

Fig. 5. Accumulation of ^{14}C-IAA in the root initials of cucumber hypocotyl cuttings. (Autoradiograms prepared from 2 and 6 days old samples)

Beyond these indirect examinations the role of cotyledons as sources of IAA, and the fact that IAA has a diversified metabolism in the young cotyledons have been proved by direct chemical analyses too.

In the cotyledons of lupine — when dry as well as at the age of 2, 4, 6 and 8 days — 13—14 different indole compounds were pointed out with TLC. These indole derivatives were found in all samples, though their quantities varied according to the age and condition of the cotyledons. Of them the following ones could be identified: tryptophan, N-malonyltryptophan, tryptamine, 5-hydroxy-indoleacetic acid, IAA, indole-3-acetonitrile, indole-3-methanol, IAA-glucosides, IAA-aspartate.

The quantitative changes of active and mobile free IAA in the cotyledons of lupine during the germination of the seed are shown in Fig. 6. As it can be seen, the free IAA is present in the cotyledons of dry seeds (0 day) in a preformed state. Its quantity increases to a considerable extent up to the 6th day of germination — certainly as a result of an induced synthesis of new IAA molecules —, then, with the cotyledons depleted, shows a marked decrease, that is the auxin is gradually transported. Thus, the auxin content of cotyledons has with all certainty an important role in germination and in the growth of the young seedling.

The concentration of free IAA increases parallel with the changes in the tryptophan (TPP) content of the cotyledons. Namely, during the rapid hydrolytic decomposition of the reserve proteins the TPP content conspicuously increases (Fig. 7). A part of the released TPP is certainly reutilized as a precursor in the IAA biosynthesis taking place in the young cotyledons.

This theory is supported also by the results of investigations made with isotope technique. Namely, if tryptophan-2-^{14}C is injected into the inflated

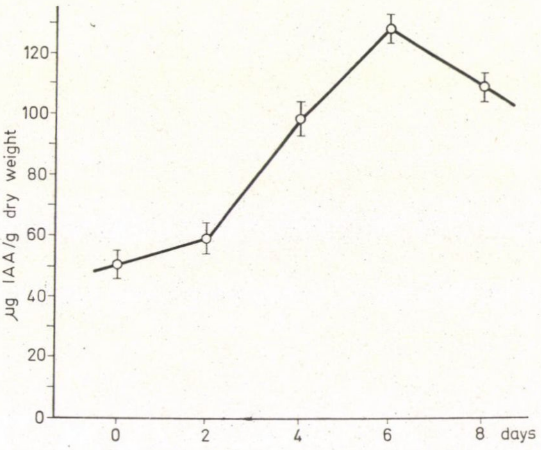

Fig. 6. Quantitative changes of free IAA in lupine cotyledons during the germination of the seed

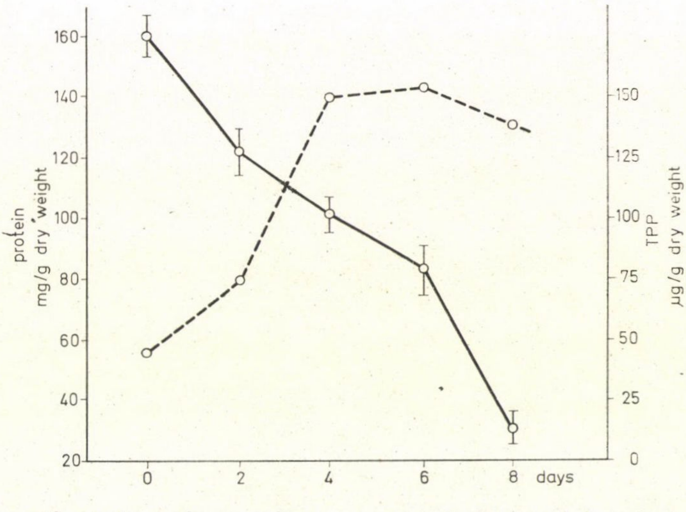

Fig. 7. Changes of protein and tryptophan contents in lupine cotyledons during germination. (— protein; - - - - - TPP)

cotyledons of lupine, radioactivity appears in a short time in various indole compounds, but mainly in IAA (Fig. 8). The cotyledons are thus really able to realize the conversion of TPP → IAA.

Of the IAA conjugates a lower amount of indole-acetyl-aspartate and a higher amount of IAA glucoside (indoleacetyl-β-D-glucose and arabinose) were found in the cotyledons of lupine (Fig. 9). Both conjugates show the highest concentration in the cotyledons of dry seeds. During the functioning of the cotyledons — while the level of free IAA considerably increases — no

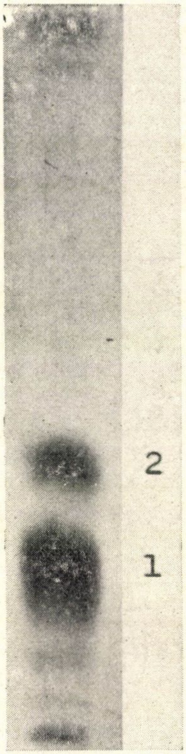

Fig. 8. Radiochromatogram made of the methanol extract of lupine cotyledons fed with TPP-2-^{14}C (1 = TPP: 2 = IAA)

new conjugates are formed, and even the initial amount decreases on the 6 –8th day of germination to a great extent.

The physiological role of the immobile IAA conjugates is a much discussed question; in various organs they may be reserved forms or precursors of IAA, and occasionally detoxification products or metabolic end-products (Varga 1968, Köves – Kovács 1972). Our results suggest that these conjugates may possibly be auxin forms stored in the cotyledons, from which IAA may be reutilized in the growth processes of the seedling according to need.

In the dry cotyledons IAA bound to macromolecules can be found too at very low concentrations (Fig. 10). In these similarly immobile complexes the IAA is bound to protein, RNA or polysaccharides (Zenk 1964, Winter – Thimann 1966, Kaur Sawhney *et al.* 1967, Varga 1968, Merkys *et al.* 1971, Davies – Galston 1971, etc.). The total amount of IAA complexes does not essentially change in the initial phase of germination, after the fourth day, however, decidedly decreases, and on the eighth day is hardly demonstrable. It seems, thus, that during the hydrolitic processes the IAA gradually becomes detached from the macromolecules and functions as an active auxin.

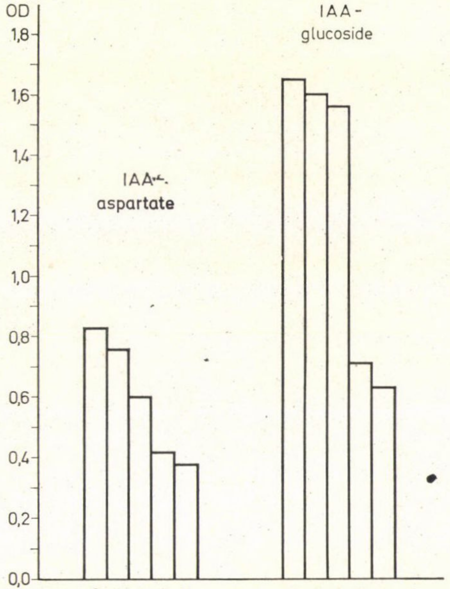

Fig. 9. Quantitative changes of IAA conjugates in lupine cotyledons during seed germination

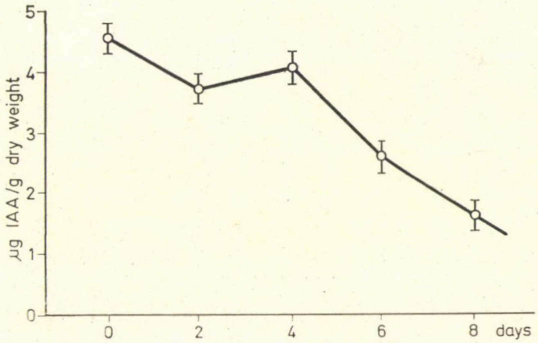

Fig. 10. Quantitative changes of IAA bound to macromolecules in lupine cotyledons during germination

For the auxin researchers the biological role of the IAA-macromolecule complexes represents a problem greater even than that of the IAA conjugates. It is probable that the role of the bound IAA too is different in the various organs (VARGA 1968). On the basis of our experiments it can be supposed that in the cotyledons the bound IAA too is one of the reserved forms of auxin. However, the IAA complexes — owing to their low amounts — are practically of no importance compared to the free acid and the IAA conjugates.

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DAMAGE CAUSED
BY TANYMECUS DILATICOLLIS GYLL.
TO LEAF AND CROP

By

A. Kacsó

RESEARCH INSTITUTE FOR PLANT PROTECTION, BUDAPEST

This paper reports on yield losses caused by the actual chewing of *Tanymecus dilaticollis* and by leaf damage modelled with mutilation, respectively. Under isolators the foliages of maize plants with 2—3 leaves are significantly damaged by more than 2, and the crops by more than 4 specimens of *Tanymecus dilaticollis* per plant. A 0.1—60-per-cent mutilation has a stimulating effect on maize plants with 2 leaves grown in glass-houses. It is characteristic of the 37 hybrids and 1 variety included in a mutilation experiment carried out in the field that they regenerate the destroyed foliage to a considerable extent, while avoiding the crop reduction is somewhat less efficient. The economic margin is a 20, percent leaf damage.

Introduction

Between 1961 and 1970 maize was produced in Hungary on an average area of 1.25 million hectares. Serious national economic interest is attached to the reliable production of maize occupying nearly 25 per cent of the arable area. Reliable production is inhibited by various pests, among others — first of all in the most important regions of maize production — by *Tanymecus dilaticollis*.

In Hungary the *Tanymecus dilaticollis* spread to a larger extent in the early fifties. Accounts of its mode of life and of the initial damage caused by this pest were given by Sáringer (1952) and Huzián (1957).

Quantitative and qualitative studies on the feeding of *T. dilaticollis* imagos were first carried out in Hungary by Sáringer (1954). According to his experiments maize plants with 2—3 leaves are completely destroyed by 66 insects/m^2. Later he found differences between the varieties as to the choice of food and intensity of leaf consumption (Sáringer—Móritz 1966). In addition to Sáringer's data Nagy (1968) reported on seedlings of 5—10 cm chewed to the ground by 50—70 insects/m^2. In dry springs the danger increases. According to Manninger sen.—Manninger jr. (1970) the *Tanymecus dilaticollis* is especially dangerous when the maize is before the one-leaf stage, though plants with several leaves, too, are hard hit by the chewing of the insect. Danger begins with 3—4 insects/m^2. Many imagos of *T. dilaticollis* cause damage to the roots of the developing plants, too. On the other hand, in experiments carried out by **Hulshoff** (1965) root pruning — or root infection analogous to it — had no essential influence on the height of plants and weight of plants and cobs. **Paulian** (1967a, b) found that the damage was the greatest in maize sown early, very early or at a time optimal for cultivation. The later the emergence, the less the damage done to the leaves. If the infection was caused by 30 insects/m^2, the destruction of leaves was 5—25 per cent at a temperature of 16—20°C, and almost 100 per cent at 18—24°C. According to **Camprag** (1969) in the case of 8 *T. dilaticollis* imagos maize plants with one leaf are destroyed in 24 hours (1 imago consumes 13 per cent of the leaf). Plants usually are attacked by more than one imagos. 50—70 imagos per m^2 are not infrequent and even 100 may occur occasionally.

The same, or nearly the same data are found in almost every author's work. However, in spite of their likeness, the above data have such deficiencies as making their practical use questionable.

The major deficiencies are:

Data — while giving the number of imagos per m^2 considered dangerous — do not indicate the number of plants per m^2, although it is far from being all the same whether the 3—4 imagos considered dangerous find 3—5 or 10 plants on an area of 1 m^2. The second deficiency is that none of the authors presents results referring to the possibility — and extent, respectively — of regeneration in completely chewed or partially damaged maize. The third deficiency is the most important. No data giving the yield losses caused by leaf damage occurring at various phenological stages are available in literature.

On the basis of the above it was only too natural to set the following research tasks:

a) to eliminate the deficiencies enumerated and

b) determine the extent of yield losses caused by various leaf damage.

Material and method

1. In order to establish the actual leaf damage caused by the *Tanymecus dilaticollis* maize was sown in a concrete ring of 0.48 m^2 area densely (25 plants/0.48 m^2) for two years, and thinly (5 plants/0.48 m^2) for two years.

On each maize plant with 2—3 leaves 2, 4, 6, 8 and 10 imagos were placed, respectively, and the plants grown in a concrete ring were isolated (Fig. 1). In the case of the densely sown maize only the leaf damage, while in that sown thinly the yield was also recorded.

The experiments were evaluated on the basis of 8 replications with variance analysis.

2. Pilot experiments on leaf mutilation. In the day-time *T. dilaticollis* usually hides and is not easy to find, so the dangerous number of insects per m^2 as an index is not readily used.

On the other hand, leaf damage caused by the *T. dilaticollis* is apparent and easy to establish either by measuring or by estimation. Our experiments were based, therefore, on leaf damage of various extent.

Leaf damage was artificially produced by the 0.1—20, 20.1—40, 40.1—60, 60.1—80, 80.1—100-per-cent mutilation of leaves

The first micro-plot mutilation experiments of pilot character were started in 1970 in the field, with six maize hybrids and the same number of plants per variation.

Mutilation mentioned above was carried out at the same time, at the 2—3-leaf- and 5-leaf stage, respectively.

Evaluation concerning the yield was performed with variance analysis, on the basis of 3 replications per variation.

3. Regeneration experiment in glass-house. Leaf damage was produced by the 0.1—20, 20.1—40, 40.1—60, 60.1—80 and 80.1—100-per-cent mutilation of leaves of potted maize plants grown in glass-house. Mutilation was carried out on two occasions, with a one-day interval, at the stage of two leaves.

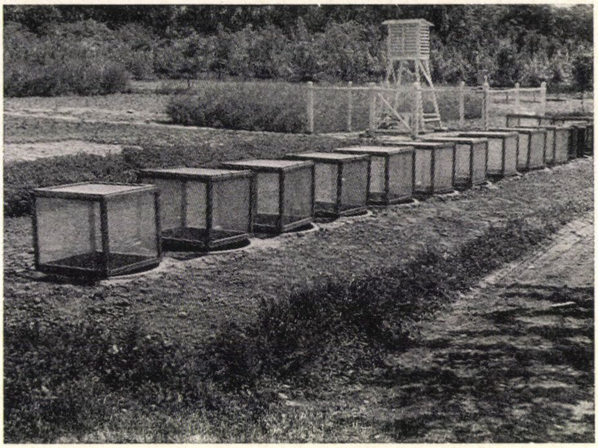

Fig. 1. Isolated concrete rings (Photo: Sáringer Dr.)

The regeneration experiments were carried out in 1971 with 4 replications, two hybrids 4, 6, 8 and 10 cm sowing depths and 4 plants per variation.

In addition to the usual control, another control was employed, namely: a repeated sowing at the time of a 100-per-cent mutilation.

When the process of regeneration following the mutilation had been completed, maize plants sown at a depth of, 4, 6 and 8 cm respectively were transplanted.

Evaluation concerning the height attained in the glass-house, the average growth vigour and the yield produced in the field were performed by variance analysis.

4. Mutilation experiment in the Julianna farm. Leaf damage was produced by the 0.1—20, 20.1—40, 40.1—60, 60.1—80 and 80.1—100- per-cent mutilation of leaves in maize sown in the field. Mutilation was carried out at the two-leaf stage on two occasions with a five-day interval.

The experiment was started in 1971, in 3 replications, with 19 hybrids and one variety sown to the same depth, with 15 plants per variation.

Similarly to the way discussed with the regeneration experiments, a second control was used, too.

Evaluation concerning plant height and yield was carried out by variance analysis.

5. Mutilation experiment at Keszthely. The method of the experiment was the same as in the Julianna farm. Differences were the following;

number of hybrids; 18,
mutilation at the stage of 2—3 leaves and 8 days later,
no second control was employed.

Results

1. Basic data of experiments set in isolated concrete rings to determine the actual leaf damage caused by the *T. dilaticollis* are contained in Table 1, while the results in Table 2.

Isolators and damage are shown by Figs 1, 2, 3, 4 and 5.

As to leaf damage results of Table 2 are based on 4 ×2 = 8, while as to the yield on 2 ×2 = 4 replications.

The mathematical evaluation of the results showed that, as for the leaf surface, there was no significant difference during the 22 days from infection to

Table 1

Basic data of experiments set to determine leaf damage done by Tanymecus dilaticollis Gyll

Designation	1966	1967	1969	1970
Site of experiment	Keszthely Isolated concrete ring	Keszthely Isolated concrete ring	Keszthely Isolated concrete ring	Keszthely Isolated concrete ring
Plant/m^2	Densely sown 25 plants/ 0.48 m^2	Densely sown 25 plants/ 0.48 m^2	Thinly sown 5 plants/ 0.48 m^2	Thinly sown 5 plants/ 0.48 m^2
Time of sowing	5 May	6 May	26 April	2 May
Placing of imagos	21 May to maize plants with 2–3 leaves	22 May to maize plants with 2–3 leaves	14 May to maize plants with 2–3 leaves	18 May to maize plants with 2–3 leaves
Evaluation				
leaf damage	14 June	13 June	6 June	10 June
yield	—	—	14 September	15 October

Table 2

Actual leaf damage and yield loss caused by Tanymecus dilaticollis

Imago number/plant	Leaf damage %	Yield %
	on 4- year average	on 2- year average
Control	0.0	100.0
2	17.0	91.0
4	41.2	107.7
6	35.2	53.7
8	57.0	35.2
10	71.7	24.2
$SD_{5\%}$	22.38	38.74

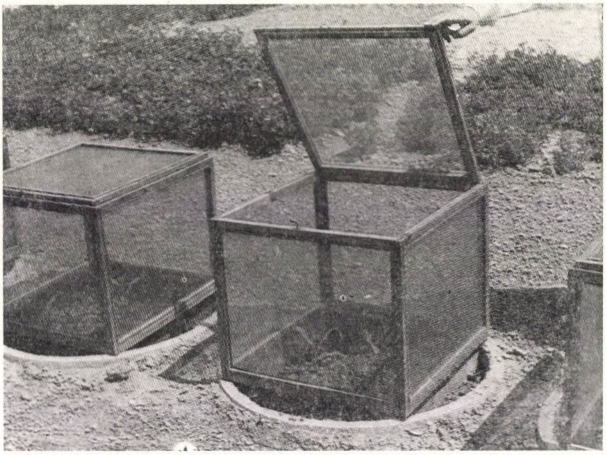

Fig. 2. Isolator with plants chewed (Photo: Sáringer Dr.)

Fig. 3. Damage done by ten imagos per plant (Photo: Sáringer Dr.)

evaluation between the control and the plants attacked by two imagos. On the other hand, 4 or more imagos per plant had a significant damaging effect on the foliage of maize affected at the stage of 2—3 leaves.

Although this experiment was aimed at determining the leaf damage only, in the last two years we succeeded in obtaining crops from the isolated plants, which made it possible to evaluate differences in yield, too.

According to the mathematical evaluation there was no significant difference in yield between the control and plants attacked by 2 and 4 imagos, respectively. On the other hand, 6 and more imagos per plant reduced the crop

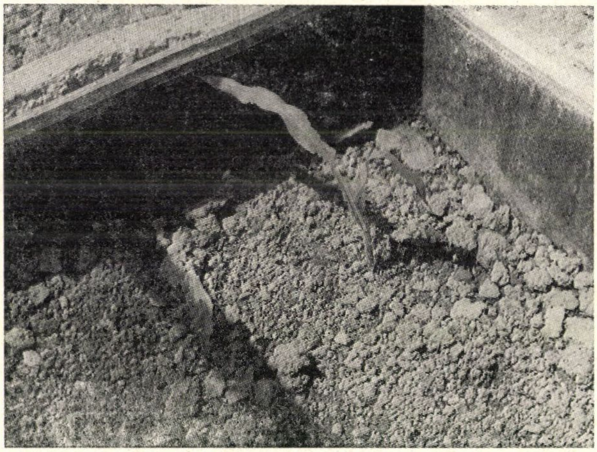

Fig. 4. Damage done by six imagos per plant (Photo: Sáringer Dr.)

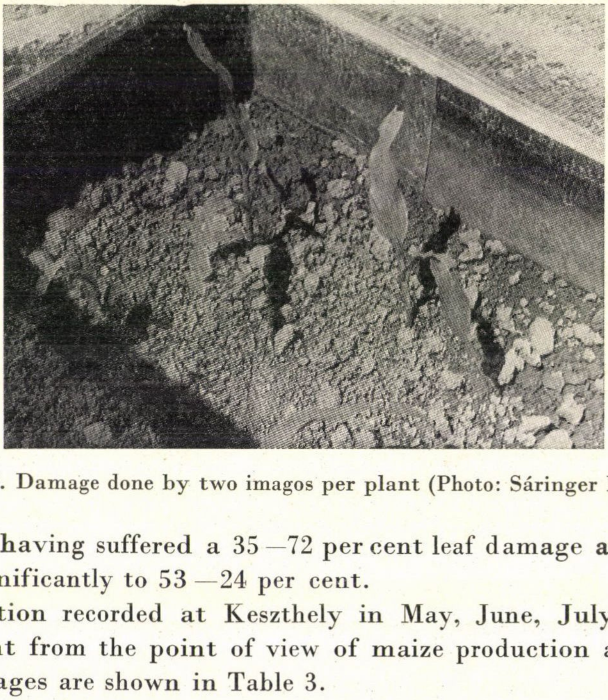

Fig. 5. Damage done by two imagos per plant (Photo: Sáringer Dr.)

yield of maize having suffered a 35—72 per cent leaf damage at the stage of 2—3 leaves significantly to 53—24 per cent.

Precipitation recorded at Keszthely in May, June, July and August, being important from the point of view of maize production as well as the fifty-year averages are shown in Table 3.

2. Basic data of our leaf mutilation pilot experiments are presented in Table 4 while their results in Tables 5 and 6.

Statistical evaluation of results related to maize plants mutilated at the stage of 2—3 leaves — as shown in Table 5 — shows that in one of the hybrids included in the pilot experiment more than 40 percent mutilation, while in the other less than 20 per cent mutilation caused a significant yield reduction.

Table 3

Precipitation amounts observed in the years of the experiment

Months	Monthly amount of precipitation (mm)											
	Budapest						Keszthely					
	1901–1950	1966	1967	1969	1970	1971	1901–1950	1966	1967	1969	1970	1971
V	68	52	59	47	33	76	74	54	88	81	28	37
VI	66	54	71	126	57	79	74	100	8	129	59	57
VII	49	111	33	50	55	48	71	190	52	25	56	47
VIII	47	84	19	54	91	20	77	42	13	111	93	16
Total	230	301	182	277	236	223	296	386	161	346	236	157

Table 4

Pilot experiments with the mutilation of maize 1970

Designation	Keszthely		Julianna farm
Site of experiment	Keszthely field	Keszthely field	Julianna farm field
Hybrid	M 5, M 59, M 520, M 530, M 570, OSK	M 59, OSK	MDC 520
Spacing	80×40 cm	80×40 cm	70×30 cm
Time of sowing	7 May	2 June	15 May
Time of mutilation	15 June at 5-leaf stage	19 June at 2—3-leaf stage	1 June at 2—3-leaf stage
Extent of mutilation	Control 0.1—20%, 20.1—40%, 40.1—60%, 60.1—80%, 80.1—100%	Control 0.1—20%, 20.1—40%, 40.1—60%, 60.1—80%, 80.1—100%	Control 0.1—20%, 20.1—40%, 40.1—60%, 60.1—80%, 80.1—100%
Evaluation yield	8 October	14 October	30 November

The mathematical evaluation of results contained in Table 6 relative to maize plants mutilated at the stage of 5 leaves shows, on the other hand, that in four of the six hybrids included in the pilot experiments mutilation above 80 per cent, while in two of them that above 40 per cent caused significant yield reductions. Precipitation amounts important from the point of view of maize production are presented in Table 3. Various degrees of yield losses found in maize plants mutilated at the 2—3-leaf- and five-leaf stages, respectively, raise the question of regeneration ability.

3. The basic data of regeneration experiments carried out in glass-houses are found partly in the section of "Material and method", partly in Table 7.

Results are shown in Tables 8, 9 and 10.

Table 5

Experiments on maize mutilation at Keszthely and in the Julianna farm, 1970
Mutilation at the stage of 2—3 leaves

Mutilation, %	Keszthely		Julianna farm
	M 59	OSK	MDC 520
	yield %		
Control	100.0	100.0	100.0
0.1— 20	97.5	91.5	113.0
20.1— 40	93.5	72.5	113.0
40.1— 60	69.5	69.5	113.0
60.1— 80	43.0	61.0	82.0
80.1—100	25.5	29.0	113.0
$SD_{5\%}$	10.08	7.47	No mathematical evaluation due to deer damage

Table 6

Experiment on maize mutilation at Keszthely, 1970
Mutilation at the stage of 5 leaves

Mutilation, %	M 5	M 59	M 520	M 530	M 570	OSK
	yield %					
Control	100.0	100.0	100.0	100.0	100.0	100.0
0.1—20	—	—	—	—	—	—
20.1—40	84.3	78.0	88.3	87.7	90.0	90.3
40.1—60	76.7	70.3	80.3	112.0	81.7	95.0
60.1—80	69.3	76.3	83.0	106.3	94.7	102.3
80.1—100	3.7	3.7	3.3	0.3	2.3	6.3
$SD_{5\%}$	16.29	73.18	18.32	32.21	33.49	23.79

Growth vigour following the mutilation was determined by measurements taken of plant heights on several occasions and averaged differences between the measurements compared to the control (Table 8).

The basic data are not suitable for variance analysis, mathematical evaluation has therefore been dispensed with. Averages show, however, that the growth vigour of plants sown to a depth of 4 and 6 cm is 2—22 per cent better than that of the control until the extent of mutilation does not exceed 60 per cent. Mutilation of leaves to 40—60 per cent almost stimulates the plants.

In plants mutilated over 60 per cent as well as in those sown to a depth of 8 and 10 cm growth vigour remains below that of the control.

In the case of control II (plants developed from a second sowing per-

Table 7

Regeneration experiments in glass-house, Budapest, 1971

Designation	Budapest	Budapest
Site	Glasshouse	Glass-house
Hybrid	MDC 520	MvTC 540
Method of sowing	Pot	Pot
Time of sowing	6 May	25 May
Depth of sowing	4, 6, 8 cm	4, 6, 8, 10 cm
Mutilation time	14 May and 15 May at 2-leaf stage mutilated twice	1 June and 2 June at 2-leaf stage mutilated twice
Mutilation extent	Control I 0.1— 20% 20.1—40% 40.1—60% 60.1—80% 80.1—100% Control II	Control I 0.1— 20% 20.1—40% 40.1— 60% 60.1—80% 80.1—100% Control II
Transplantation in the field	4 June	—
Evaluation	Growth vigour Height Yield	Growth vigour Height

Table 8

Mutilation of maize at the 2-leaf stage in glass-house Budapest, 1971. Average percentage growth vigour

Mutilation, %	Depth of sowing, cm			
	4	6	8	10
Control I	100.0	100.0	100.0	100.0
0.1—20	115.1	106.5	97.5	84.9
20.1—40	114.7	102.4	100.4	84.0
40.1—60	109.4	121.9	98.4	101.1
60.1—80	97.3	80.3	94.5	87.5
80.1—100	61.1	102.8	71.6	77.1
Control II	103.6	94.0	79.8	68.5

formed simultaneously with the 100 per cent mutilation) the growth vigour is the poorer, the deeper the seeds have been sown.

As regards height measured for the last evaluation, with maize plants sown to a depth of 4, 6 and 8 cm, significant difference could be found only with the largest extent (60 —80 and 80 —100 per cent respectively) of mutilation (Table 9).

Heights of plants sown to a depth of 10 cm show such a high degree of dispersion that $SD_{5\%} = 0.0$. The differences are not significant.

Table 9

Mutilation of maize at the 2-leaf stage in glass-house Budapest, 1971. Percentage height in the last evaluation

Mutilation, %	Depth of sowing, cm			
	4	6	8	10
Control I	100.0	100.0	100.0	100.0
0.1—20	105.0	94.5	95.0	79.0
20.1—40	106.0	97.5	94.0	65.5
40.1—60	93.5	100.5	87.0	78.0
60.1—80	81.5	66.5	81.0	68.0
80.1—100	42.5	72.0	58.0	53.0
Control II	61.5	56.0	53.5	39.0
$SD_{5\%}$	25.14	27.27	32.46	0.0

Table 10

Maize plants mutilated at 2-leaf stage in glass-house then transplanted in the field, Budapest, 1971 Yield, plant/kg, expressed as a percentage to control I

Mutilation, %	Depth of sowing, cm			
	4	6	8	10
Control I	100.0	100.0	100.0	not transplanted
0.1—20	155.2	69.5	74.5	
20.1—40	118.0	130.5	57.7	
40.1—60	140.7	83.0	78.0	
60.1—80	124.2	63.5	72.5	
80.1—100	76.0	115.0	87.2	
Control II	123.0	133.7	108.5	
$SD_{5\%}$	0.0	0.0	24.8	

Crop results of maize plants transplanted after regeneration processes following the mutilation had been completed in the greenhouse are shown in Table 10.

Mutilation and adaptation to the new conditions caused such dispersion in the yield that in maize plants sown to a depth of 4 and 6 cm no significance was found ($SD_{5\%} = 0.0$). Apart from the mathematical evaluation yield results of control II suggest the same. In one of the variations of maize sown to a depth of 8 cm significant difference is acceptable while in two variations of it it is but a minimum. These three significance values have, however, quite illogical places in the data series of mutilation and crop results.

Table 11

Experiment on maize mutilation in the field, Julianna Farm, 1971
Height percentage in the last evaluation

Hybrids	Mutilated twice, sown to a depth of 4–6 cm						
	Percentage extent of mutilation						
	Control I	0.1–20	20.1–40	40.1–60	60.1–80	80.1–100	Control II
Mv 26	100	97	100	100	101	96	58
MvDC 5	100	107	91	108	100	82	66
MvDC 59	100	102	98	94	99	97	62
MvDC 520	100	106	101	105	104	99	71
MvMC 40	100	97	97	91	98	92	67
MvSC 530	100	102	100	98	100	98	70
MvSC 570	100	94	96	96	97	94	69
MvSC 660	100	92	92	89	92	79	62
MvTC 290	100	96	93	91	97	91	67
MvTC 540	100	103	99	103	104	97	69
MvTC 610	100	101	104	98	102	90	76
Black m.	100	111	104	92	113	94	65
GDC 250	100	105	104	99	98	99	84
GTC 304	100	100	102	103	101	95	62
K 360	100	94	97	92	86	88	51
K 532	100	106	101	105	106	92	74
K 583	100	103	100	100	101	92	70
KTC 502	100	102	110	106	108	85	73
GKI	100	97	90	99	99	98	57
DC 364 A	100	92	95	96	92	92	75
Average	100.0	100.4	98.7	98.3	99.9	92.5	67.4

Precipitation amounts measured in periods important from the point of view of maize transplanted in the field, are presented in Table 3.

4. Mutilation experiments were carried out in the Julianna farm with 19 hybrids and 1 variety of maize. Sowing was performed on 17th May to a depth of 4–6 cm, with a spacing of 50 ×35 cm. Mutilation was carried out on two occasions, first on 3–4 June, the second time on 8 June. The amounts of precipitation are contained in Table 3.

Since the main object of research was to determine the yield losses caused by the various degrees of leaf destruction, trends in the heights of plants were evaluated by averaging and frequency calculations only.

As for the heights of plants the results of the experiment are shown by Table 11.

Fig. 6. Hybrid Mv 26 mutilated to various degrees and re-sown (Control II) (Photo: Kacsó Dr.)

Fig. 7. Hybrid MvDC 5 mutilated to various degrees and re-sown (Control II) (Photo: Kacsó Dr.)

Data of Table 11 demonstrate sufficiently — even without variance analysis — the different regeneration abilities of the hybrids.

Of the mass of data, however, the height values of control II are the most interesting, being in every case lower than those of variations mutilated to 80—100 per cent. This means that maize plants out off at ground level at the two-leaf stage regenerate and will be more vigorous than those developing from seed sown simultaneously with the mutilation (Figs. 6 and 7).

In Table 12 frequency of heights deviating from those in control I are shown. Frequency of occurrence of plants with the same height as that of control I, or higher than that proves regeneration ability in most hybrids. Maize mutilated to 80—100 per cent, with results better than those in control II is an exception.

Table 12

Mutilation of maize in the field, Julianna farm, 1971
Frequency of heights deviating from control I

Mutilation, %	1	2	3	2 and 3
	lower below 95%	practically identical between 95 and 105%	higher above 105%	together
	frequency %			
Control I	0	100	0	100
0.1—20	20	55	25	80
20.1—40	20	75	5	80
40.1—60	30	50	20	70
60.1—80	15	70	15	85
80.1—100	60	40	0	40
Control II	100	0	0	0

Table 13 shows the yield results expressed as a percentage of control I and mathematically evaluated by variance analysis.

With four hybrids and one variety the yield results show a high degree of dispersion ($SD_{5\%} = 0.0$). Differences are not significant. In the case of the other hybrids with mutilations of various extent yield differences are significant in 4—8 cases.

Table 14 shows the frequency of crop results deviating from control I.

Frequency of crop results practically identical with and higher than those of control I —to 80—100-per-cent mutilation — is 20—50 per cent, while with a mutilation of 80—100 per cent only 15 per cent. Thus data of Tables 12 and 14 show that maize hybrids included in the experiment are able to regenerate the vegetative parts to a considerable extent, while from a generative aspect the efficiency of regeneration is low.

5. The mutilation experiment at Keszthely was carried out with 18 maize hybrids. Sowing was performed on 7 May to a depth of 4—6 cm with a spacing of 70 ×40 cm. Mutilation was carried out on two occasions, first on 22 May, the second time on 30 May. Precipitation amounts are shown in Table 3.

Trends in the heights of plants were evaluated by averaging and with frequency calculations only. Results of the experiments concerning plant height are contained in Table 15.

Height results obtained in the mutilation experiment at Keszthely reflect similar trends as those in the Julianna farm. Regeneration ability in the individual hybrids, though decidedly recognizable, is of different degree.

In Table 16 frequency of heights deviating from the control is presented. Frequency of occurrence of plants of the same height as and higher than the

Table 13

Maize mutilation in the field in the Julianna Farm, 1971
Yield plant/kg, expressed as a percentage to control I

Hybrids	Percentage extent of mutilation							SD $_{5\%}$
	Control I	0.1–20	20.1–40	40.1–60	60.1–80	80.1–100	Control II	
Mv 26	100.0	62.3	84.6	80.6	75.0	82.3	19.3	25.5
MvDC 5	100.0	83.6	53.0	87.0	85.0	50.6	13.0	32.4
MvDC 59	100.0	85.3	77.3	71.0	76.7	57.0	10.3	22.5
MvDC 520	100.0	83.0	73.0	80.0	71.3	75.0	23.0	21.3
MvMC 40	100.0	86.6	84.6	81.0	128.3	79.0	29.0	37.1
MvSC 530	100.0	106.0	95.3	87.6	110.3	91.6	18.6	33.2
MvSC 570	100.0	91.3	93.3	95.3	97.0	107.6	34.0	22.6
MvSC 660	100.0	94.3	64.3	58.3	82.6	56.6	—	29.4
MvTC 290	100.0	135.3	75.3	79.3	107.6	78.3	35.3	0.0
MvTC 540	100.0	147.0	111.6	115.6	142.3	123.6	56.3	0.0
MvTC 610	100.0	56.6	69.3	73.3	103.0	77.0	20.0	28.6
Black m.	100.0	94.3	125.6	77.6	134.3	75.0	—	0.0
GDC 250	100.0	126.3	104.6	103.6	118.6	119.6	63.3	18.7
GTC 304	100.0	73.0	65.3	72.0	82.6	53.0	18.0	15.4
K 360	100.0	70.0	62.3	54.3	66.3	60.0	22.0	17.3
K 532	100.0	86.0	84.6	90.6	100.3	83.6	35.3	22.3
K 583	100.0	96.6	56.0	100.6	83.6	78.6	25.0	34.4
KTC 502	100.0	167.0	131.3	90.3	128.6	66.6	17.0	0.0
GKI	100.0	85.3	91.6	81.6	80.0	86.3	45.0	20.9
DC 364 A	100.0	74.3	62.6	79.6	63.0	81.0	60.3	0.0
Mean	100.0	95.2	83.3	83.1	96.9	79.5	30.2	

control proves the regeneration ability of most of the hybrids. Maize mutilated to 80–100 per cent is an exception.

Crop results expressed as a percentage proportion of the control and evaluated mathematically by frequency analysis are shown in Table 17.

In ten hybrids the yield results are highly dispersed ($SD_{5\%} = 0.0$). Differences are not significant. In eight hybrids, with mutilations of various extent, yield differences are significant in 3–4 cases, while in two hybrids in 1–2 cases.

Table 18 shows the frequency of crop results deviating from the control. Frequency of crop results practically identical with and higher than those of the control is to a 80–100-per-cent mutilation 28–55 per cent, while with a mutilation of 80–100 only 12 per cent. As to their order of magnitude these data correspond to results obtained in the experiment on the Julianna farm.

Table 14

Maize mutilation in the field, Julianna Farm, 1971
Frequency of crop results deviating from control I

Mutilation, %	1	2	3	2 and 3
	lower below 95%	practically identical between 95 and 105%	higher above 105%	together
	frequency %			
Control I	0	100	0	100
0.1—20	70	5	25	30
20.1—40	75	10	15	25
40.1—60	80	15	5	20
60.1—80	50	15	35	50
80.1—100	85	0	15	15
Control II	100	0	0	0

On the basis of the uniform results it seems — here again — that the regeneration of the vegetative parts is much more efficient than that of the generative parts.

Conclusions

From the results of experiments carried out in five years the following conclusions can be drawn:

1. The leaf surface of hybrid maize artificially infected at the 2—3-leaf stage and raised under isolator is damaged only by more than 2 imagos per plant, while the ears only by more than 4 imagos per plant. With less imagos than that per plant no significant differences are found between the control and the affected leaves and ears of the maize, respectively.

4—10 imagos per plant destroy 41—72 per cent of the leaf surface, while 6—10 imagos 46—76 per cent of the crop.

2. The growth vigour of maize plants sown in glasshouse to a depth of 4 and 6 cm and mutilated to 0.1—60 per cent at the two-leaf stage is improved by 2—22 per cent.

In the case of mutilation above 60 per cent, as well as when plants are sown deeper, depression occurs.

In case maize plants are sown to a depth of 4, 6 and 8 cm respectively, significant differences are found in heights of plants mutilated above 60 per cent only.

Yields of maize transplanted from the glass-house into the open are so much dispersed that they are not suitable to draw conclusions from.

Table 15

Field experiment with maize mutilation at Keszthely
Height percentage in the last evaluation, 1971

Hybrids	Percentage extent of mutilation					
	control	0.1–20	20.1–40	40.1–60	60.1–80	80.1–100
Mv 59	100	91	86	87	83	62
Mv 520	100	98	100	101	101	51
Mv 530	100	105	107	113	92	77
Mv 570	100	97	105	99	99	66
G 602	100	82	75	72	99	71
GDC 250	100	93	79	86	86	86
GSC 360	100	89	71	94	96	78
GSC 361	100	99	115	113	99	94
GTC 277	100	79	74	84	85	79
K 22	100	108	107	106	112	93
K 364	100	106	95	103	101	99
K 368	100	100	102	102	106	100
K 503	100	116	122	123	117	86
K 597	100	113	108	114	111	105
KDC 341	100	120	123	124	93	91
KDC 345	100	91	98	102	98	91
ZPSK 6	100	101	101	95	93	75
ZTSK 46	100	97	108	105	99	89
Mean	100	99	99	101	98	83

Table 16

Mutilation of maize in the field at Keszthely
Frequency of heights deviating from the control, 1971

Mutilation, %	1	2	3	2 and 3
	lower below 95%	practically identical between 95 and 105%	higher above 105%	together
	frequency %			
Control	0	100	0	100
0.1—20	33	39	28	67
20.1—40	28	33	39	72
40.1—60	28	39	33	72
60.1—80	33	45	22	67
80.1—100	83	17	0	17

Table 17

Mutilation of maize in the field at Keszthely
Yield plant/kg, expressed as a percentage to the control, 1971

Hybrids	Percentage extent of mutilation						SD $_{5\%}$
	control	0.1–20	20.1–40	40.1–60	60.1–80	80.1–100	
Mv 59	100.0	68.6	66.0	32.6	59.3	20.0	33.9
Mv 520	100.0	88.2	75.8	71.7	60.3	25.7	14.6
Mv 530	100.0	79.8	87.0	85.8	74.3	45.2	21.6
Mv 570	100.0	84.3	82.3	69.5	72.5	54.1	28.6
G 602	100.0	58.6	44.0	40.3	100.6	23.0	51.5
GDC 250	100.0	81.0	101.3	56.6	48.0	35.3	43.9
GSC 360	100.0	108.0	27.0	60.0	67.0	32.6	32.9
GSC 361	100.0	130.0	138.0	114.3	56.6	—	0.0
GTC 277	100.0	98.0	92.0	111.6	99.6	20.8	54.4
K 22	100.0	152.3	134.0	170.6	127.6	106.6	0.0
K 364	100.0	127.3	103.0	85.0	74.6	77.6	0.0
K 368	100.0	144.0	129.6	101.6	97.0	98.3	0.0
K 503	100.0	139.0	140.6	84.3	101.0	38.0	0.0
K 597	100.0	107.0	114.0	113.3	84.3	77.6	0.0
KDC 341	100.0	115.0	105.0	115.6	80.3	—	0.0
KDC 345	100.0	99.6	101.3	97.3	89.6	64.6	0.0
ZPSK 6	100.0	86.6	75.0	58.6	38.0	23.0	0.0
ZTSK 46	100.0	83.6	92.3	86.3	87.6	46.6	0.0
Mean	100.0	102.8	94.9	86.4	78.8	49.3	

Table 18

Mutilation of maize in the field.
Frequency of crop results deviating from the control,
Keszthely 1971

Mutilation, %	1	2	3	2 and 3
	lower below 95%	practically identical between 95 and 105%	higher above 105%	together
	frequency %			
Control	0	100	0	100
0.1–20	45	11	44	55
20.1–40	50	22	28	50
40.1–60	61	11	28	39
60.1–80	72	22	6	28
80.1–100	88	6	6	12

In general we can say that in case the life conditions of maize are provided for in a glass-house, low and medium degrees of mutilation have a stimulating effect on the growth vigour and height of maize. Thus, under certain conditions maize plants are able to replace through their regeneration ability the vegetative parts lost.

This statement is proved by the regeneration field experiment carried out in the Julianna farm too, where maize plants cut at ground level (100 per cent mutilated) at the two-leaf stage became 16—45 per cent stronger than those developed from a second sowing (control II).

3. Mutilation experiments of informative character carried out in the Julianna farm and at Keszthely equally prove that the crop results of the different hybrids are highly dispersed. Considering that besides mutilation the productive and regenerative ability of the hybrids also take part — among others — in the development of the yield, results can be generalized "for the maize" only with reservation. The results apply first of all to the individual hybrids.

On the other hand, the conclusion that the hybrids are highly efficient in regenerating the destroyed foliage but less able to prevent the reduction of yield can be safely drawn as a characteristic of them.

In the experiments carried out in the Julianna farm and at Keszthely crop results changed identically under the influence of mutilation.

In the Julianna farm frequency of crop results identical with or higher than that of the control is — up to a 80-per-cent mutilation — 20—25 per cent, while with total mutilation only 15 per cent.

At Keszthely frequency of crop results identical with or higher than that of the control is — up to 80 per cent — 28—55, while in the case of a total mutilation only 12 per cent.

The harmfulness of *T. dilaticollis* should be determined by the assessment of leaf damage found in the maize rather than on the basis of the number of imagos per m^2.

Considering that of the 37 hybrids and one variety included in the experiment it was only in 4 cases that a 0.1—20 per cent leaf mutilation carried out at the 2—3-leaf stage caused a low extent of significant yield loss, this damage need not be considered dangerous.

A leaf damage of 0.1—20 per cent — when characterized by the number of imagos per m^2 — corresponds to 3—6 imagos per m^2 in the case of 3 plants per m^2 and to 4—8 imagos per m^2 with 4 plants per m^2.

A leaf damage of 20—40 per cent observed at the 2—3-leaf stage is already dangerous ! Although in most cases maize plants recover from the leaf damage, a frequent significant yield loss of more than 10 per cent should be reckoned with.

This means that when leaf damage attains 20 per cent, protection must be started with a view to the reliability of production!

It should be noted that in the case of significant yield losses a protection cost of 190 Ft/ha — calculated with 1971 prices — will be repaid, therefore the 20 per cent leaf damage can be considered an economic limit value (Kacsó 1972).

Maize plants must not be turned up in a hurry even in the case of a high-extent (80—100 per cent) leaf damage observed at the 2—3-leaf stage, and re-sowing must be thoroughly considered, as maize plants with two leaves chewed to the ground (mutilated) will develop after the destruction of imagos into plants stronger than those grown from a second sowing.

Proposals on the assessment of the harmfulness of *T. dilaticollis* and on the timeliness of control to be carried out, as well as those connected with the problem of re-sowing, are primarily of economic importance because they serve the reliable production of maize on one hand, and outline the bases of a profitable plant protection activity, on the other.

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DAILY AND SEASONAL CHANGES OF SOLUBLE CARBOHYDRATES IN ANDROPOGON GAYANUS (NORTHERN GAMBA GRASS)

By

A. A. ADEGBOLA, E. BALOGH

DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF IFE, ILE-IFE, NIGERIA

The common occurrence of *Andropogon gayanus* in the natural grasslands of Nigeria suggested the study of its nutritional value, the changes in the physiological factors affecting its growth. The authors studied the quantitative changes of the soluble carbohydrates and starch in the different plant parts, as the main sources of energy reserve in Gamba grass. The experimental plants were grown on the Farm of the University of Ife. Samples were taken at regular intervals between June 1969 and June 1970. The separated plant parts were analysed for sugar and starch content according to Block and Pucher et al. It was found that sucrose, glucose and fructose were always present in varying quantities in the different plant parts. Sometimes maltose, xylose, arabinose also occurred in small quantities. The character of the changes is discussed in detail in the paper presented for publication. Similarly, the changes in starch content during growth are also discussed. As a conclusion, we have found no direct connection between the production of carbohydrates during photosynthesis and the concentration of free sugars in the plant. On the basis of the literature and our results there is a further need for the study of those regulation systems which influence the nutritional value of Gamba grass and its use for good- quality silage.

Introduction

Andropogon gayanus (Northern Gamba grass) is a common species occurring in natural grasslands in many parts of the country (BOWDEN 1963, MCKELL—ADEGBOLA 1966, HAGGAR 1970). The authors studied the seasonal and daily changes in the carbohydrate and starch content of Northern Gamba grass, because the carbohydrates are the main sources of the energy reserve, used during the growth of the grass. In the economical use of the plant for fodder it may be important to know the changes in the physiological factors affecting its growth, as such information might be useful in production. In these experiments, the changes of the soluble carbohydrates and starch in the plant parts of *Andropogon gayanus* were determined quantitatively and the effects of these data on the nutritive and agricultural use of this species are discussed.

Agriculturally valuable grasses may be classified into two groups depending on the chemical compounds present in the reserved organs. Tropical grasses have starch and sucrose in their reserved organs, while temperate grasses largely contain fructosans (DECUGNAC 1931, WEINMANN—REINHOLD 1946). MCILROY (1967) points out that the various nutrients in the reserved organs are used up in different periods during growth, the reducing sugars are utilized in the vegetative period, sucrose is largely used during the period of differentiation and other preserved polysaccharides during secondary growth. Further characteristic changes occur in the quantity of carbohydrates which is lower during the vegetative period and higher during ripening (WEINMANN 1961).

The main sugars found in grasses are sucrose, glucose, fructose and polysaccharide. ADEGBOLA—MCKELL (1966) indicated the importance of sucrose as a substrate for the enzymatical formation of reserve polysaccharides.

Material and method

The plants used in this experiment were grown from seed on the Experimental Farm of the University of Ife. The first samples were taken in June 1969, six weeks from the time of seeding. Further samples were taken approximately every fortnight till the middle of June 1970. All samples were taken between 9 and 10 a.m. The plants were separated into leaves, stem and root fractions and were fixed immediately in 80-per-cent boiling ethanol. The soluble carbohydrates were extracted for two hours with 80-per-cent ethanol in a refluxing extractor (Labconco). The extracts were made up to equal volumes. The soluble sugars were separated chromatographically on Whatman No. 1 paper. For a better separation the chromatograms were developed three times in butanol-acetic acid-water 4 : 1 : 1 mixture. For the identification of the position of the sugars on the chromatograms benzidine-trichloroacetic acid-ethanol reagent was used. After this identification the respective sections from the undeveloped strips were extracted with distilled water for 24 hours. The sugars were determined from these extracts with 0.2-per-cent anthrone sulphuric acid reagent. The colour was measured spectrophotometrically (Beckman DB) at 620 mu.

All analytical data were calculated on a dry-weight basis. The dry-weight content was measured on parallel samples dried at 105 °C for 24 hours. In those experiments where the daily changes of sugar contents were measured, samples were taken every third hour through 24 or 48 hours. These samples were immediately fixed in boiling 80-per-cent ethanol, and were later analysed as previously described.

For the determination of the starch in the plant parts the method outlined by PAECH—TRACEY (1955) was employed. The samples were extracted in hot acetone to remove plant pigments and lipids. The extracted material was dried at 50 °C and grated on Glen Creston mill. 500 g of the ground material was used for perchloric acid extraction. For the preparation of the starch-iodine complex 20-per-cent NaCl and iodine-KJ reagent was used. The samples were kept in a refrigerator for 24 hours in closed test tubes to obtain a precipitate. In the original methods, the recommended precipitation time was 20 minutes, but the low starch content of these samples necessitated a longer precipitation time. In the decomposition of the starch-iodine complex and the hydrolysation of the starch we followed the original procedure. We used 1N oxalic acid instead of 0.1N for discharging the neutralized hydrolysate's colour. Finally, the sugar content was determined by Somogyi reagent and with 1N sodium thiosulphate solution. The percentage of starch was calculated with the aid of the following equation:

$$S = 0.90 \times \frac{5.000\,VG}{WEA}$$

where S is the percentage of starch, W is the weight of the sample in mg, E is the volume of perchloric acid extract taken, V is the volume of starch hydrolyzate, A is the aliquot of the starch hydrolyzate taken, G is the number of mg of glucose in A and 0.90 is the theoretical factor for converting glucose to starch.

Results

It was found that sucrose, glucose and fructose are always present in varying quantitatives in the different parts of Northern Gamba grass. Sometimes, maltose, xylose and arabinose also occur in small quantities or in traces in every plant part.

During the growing period, the above-mentioned sugars show great variability. In the young, 6-weeks-old plants all of the sugars are present at a high concentration, especially in the stem. During the vegetative period small decreases occur, and the decrease is continuous until flowering. In full bloom, the quantity of the sugars increased considerably in all of the examined plant parts. The sucrose content was high in the roots, while the sucrose and glucose were present in greater quantities in the leaves (Figs 1, 2, 3). The quantity of fructose had changed slightly. After flowering the sugar content gradually decreased. Following cutting, and during the new growing period, the percentage of sucrose was high in all plant parts. The glucose content was also high, and the fructose content increased, in relation to the previous stage. The second prebloom shows slight decreases in the soluble sugar content in the leaves and stems, and significantly less in the roots. During the second bloom the sugars were present in a higher concentration (0.8 to 1.21 per cent) in the leaves and stem, while the roots showed a much lower sugar content (Figs 1—3).

For the examination of the daily changes, three series of samples were taken, the first in the eighth week, the second during the twenty-fourth week, and the third during the forty-second week of the experiment.

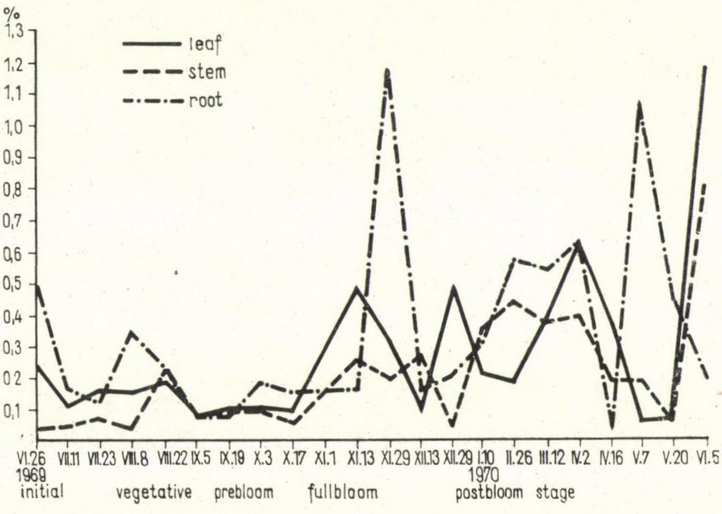

Fig. 1. **Seasonal changes of sucrose in Gamba grass**

In the young vegetatively growing plant the maximum sugar content was in the late afternoon and in the night hours, before midnight. The sugar content was low in all fractions collected in the morning hours (Figs 4, 5, 6).

In the second experiment made during the flowering period the lowest values for sugar contents were obtained in the later afternoon hours, early evening. During the morning hours the sugar content was at its highest level (Figs 7, 8, 9).

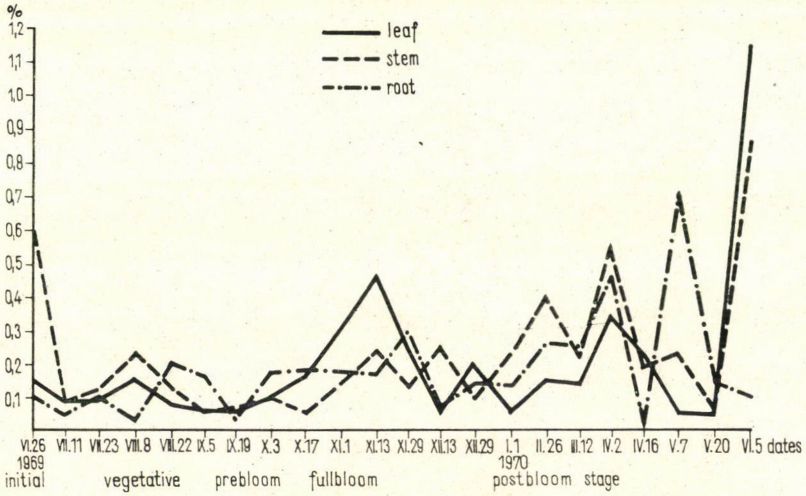

Fig. 2. Seasonal changes of glucose content in Gamba grass

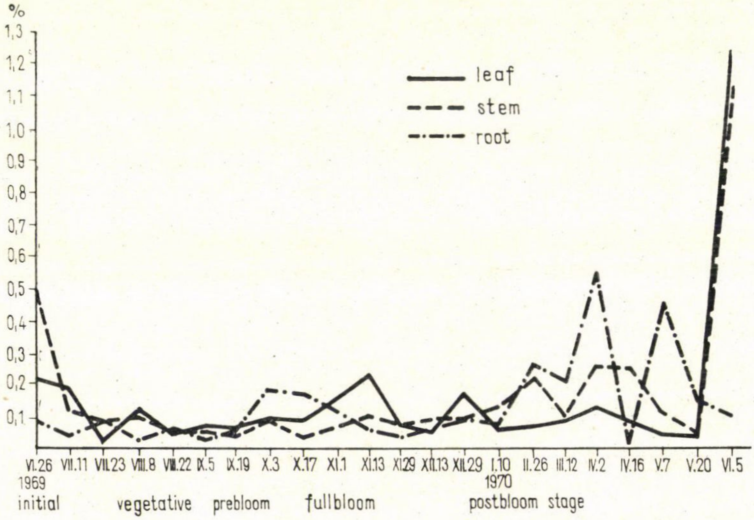

Fig. 3. Seasonal changes of fructose content in Gamba grass

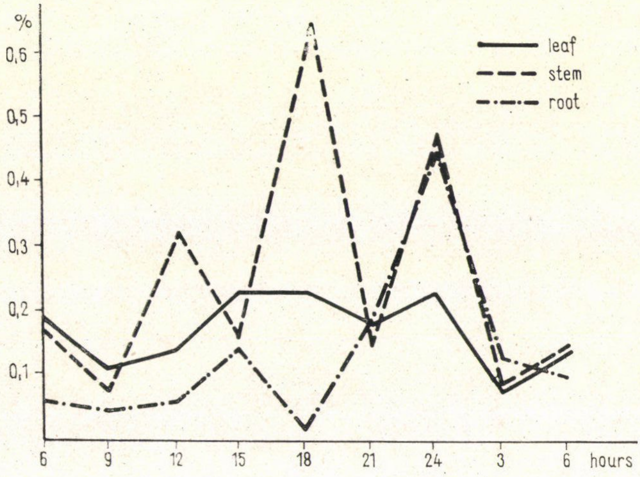

Fig. 4. Daily changes of sucrose in Gamba grass

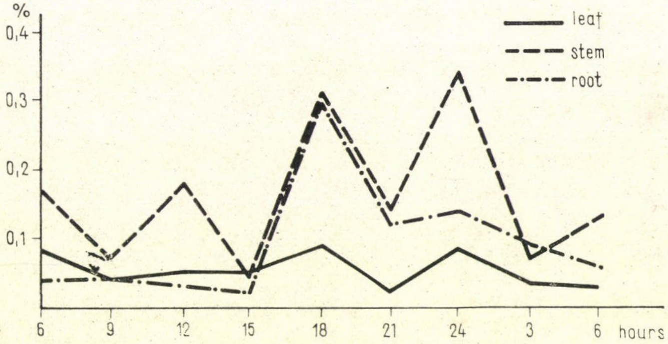

Fig. 5. Daily changes of glucose in Gamba grass

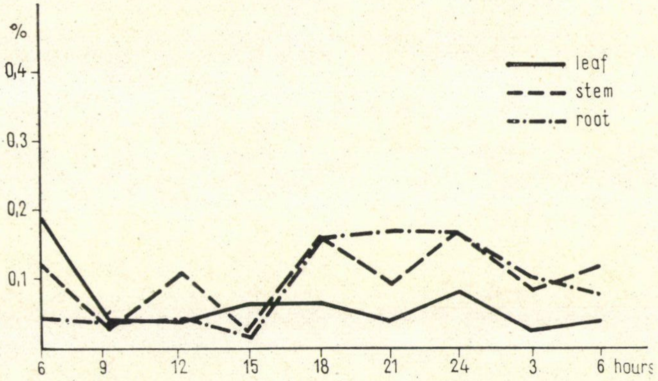

Fig. 6. Daily changes of fructose in Gamba grass

In the third experiment, at the post-bloom stage, the daily changes of sugars was examined through 48 hours. At first, the highest sugar content was in the stem, while in the leaves and roots it increased gradually. In the leaves the sugars increased in the late afternoon and evening. During the night, a decrease was characteristic. The sugar content was highest in the early morning hours in the roots. Similar changes were observed on the second day of sampling, but the percentage was somewhat higher (Figs 10, 11, 12).

In parallel experiments we studied the changes of starch in Northern Gamba grass. During the initial growing period, a relatively high starch content was observed in the leaves which decreased as fullbloom was reached. After flowering, cutting, and in the repeated growing stage, the starch content

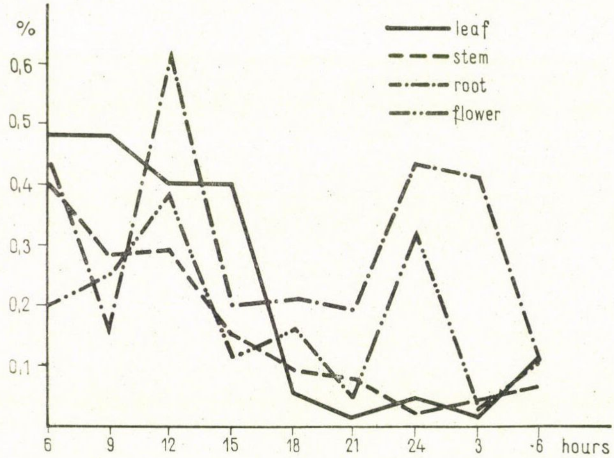

Fig. 7. Daily changes of sucrose in Gamba grass

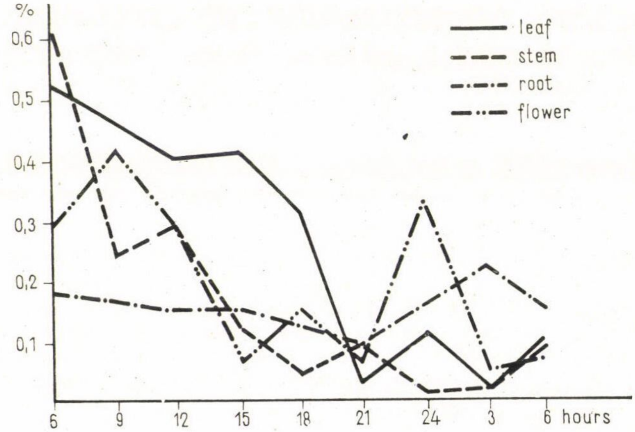

Fig. 8. Daily changes of glucose in Gamba grass

remained below 1 per cent. In the stem and roots, the changes of starch were more proportional, but in value lower than in the leaves. The starch content in the roots increased after cutting and prior to the second flowering (Fig. 13).

Daily changes of starch in the leaves during the vegetative stage showed a high starch percentage in the early morning hours, which decreased gradually. The starch content increased again by the late afternoon. In the stem the changes were less characteristic. The starch content at noon and early morning was high in the roots (Fig. 14).

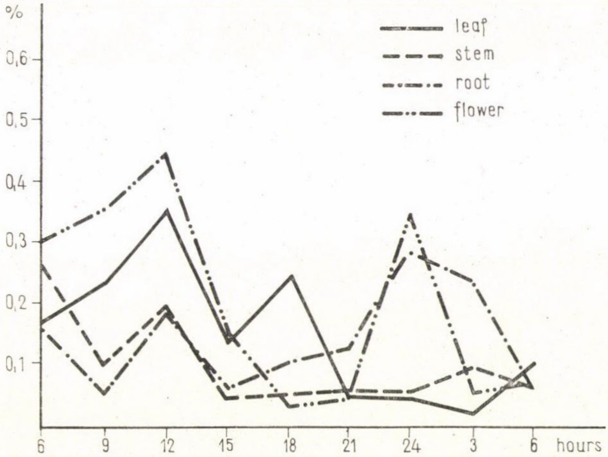

Fig. 9. Daily changes of fructose in Gamba grass

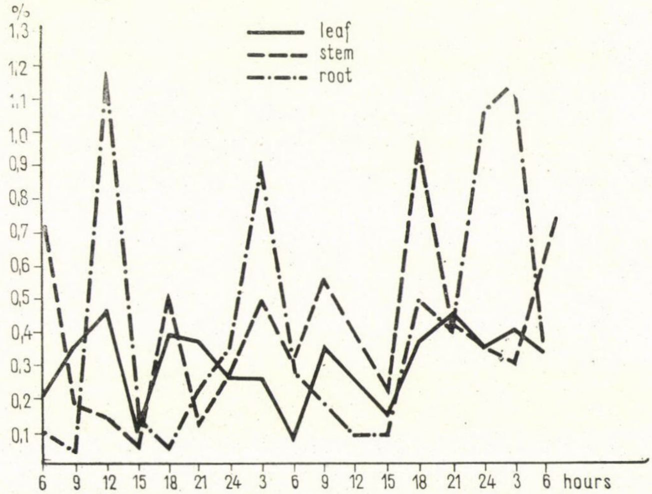

Fig. 10. Changes of sucrose content in Gamba grass during the 48-hour experiment

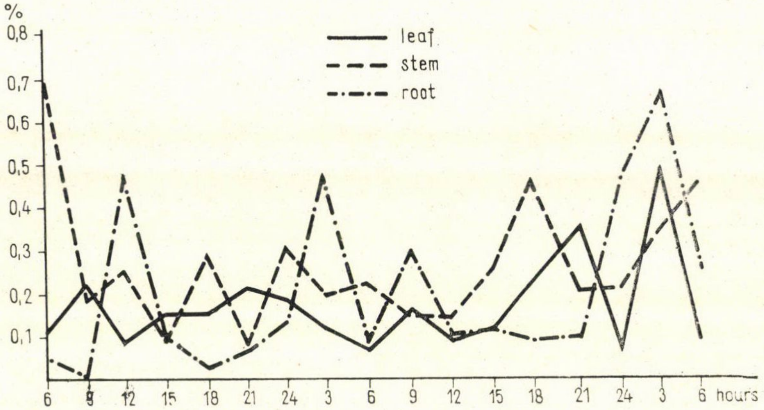

Fig. 11. Changes of glucose content in Gamba grass during the 48-hour experiment

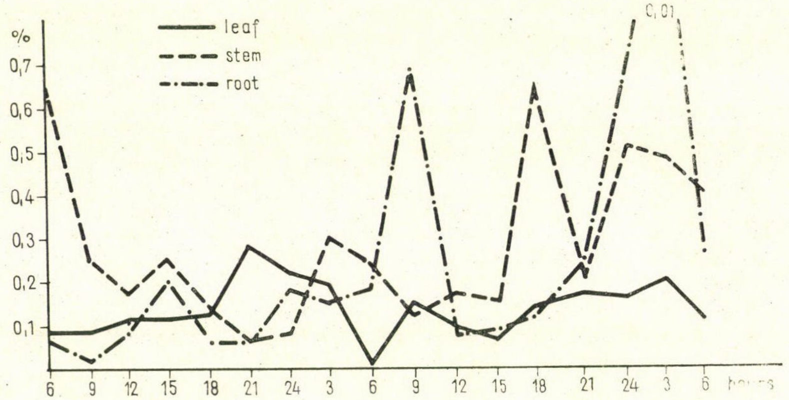

Fig. 12. Changes of fructose content in Gamba grass during the 48-hour experiment

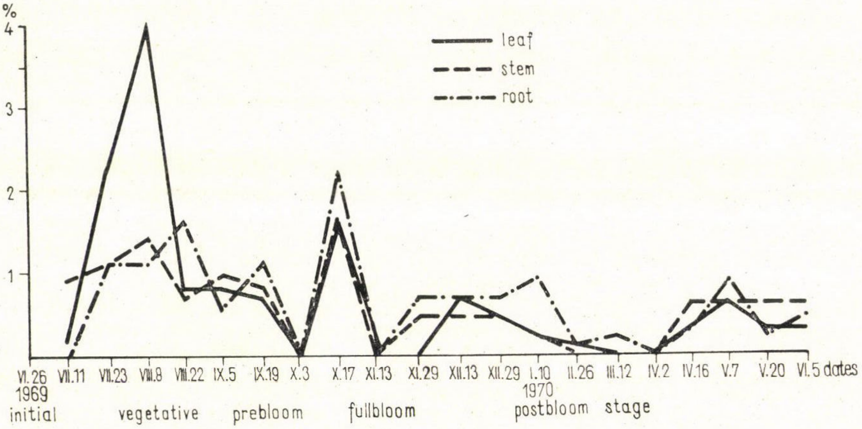

Fig. 13. Seasonal changes of starch content in Gamba grass

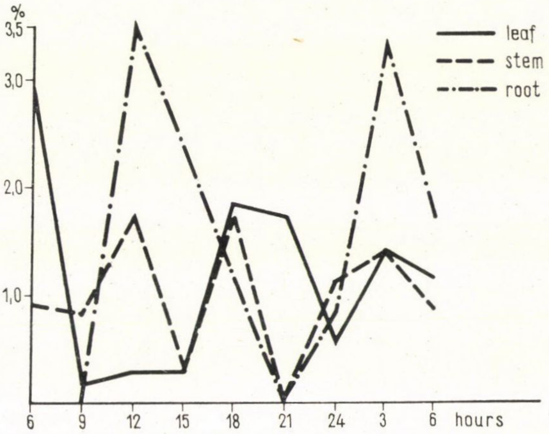

Fig. 14. 1st exp. Daily changes of starch in Gamba grass

During fullbloom, in the second experiment, the starch content was low in all parts of the plants. The starch content increased in the late afternoon hours in the leaves, while during the night the accumulation was higher in the stem, during the morning and night the percentage of starch increased in the roots (Fig. 15).

In the third, 48- hour experiment, we observed a high starch content in the afternoon and evening in the leaves. At noon the starch content was low in the stem, and the changes during the day were relatively small. The starch content was considerably higher in the morning and late night in the roots (Fig. 16).

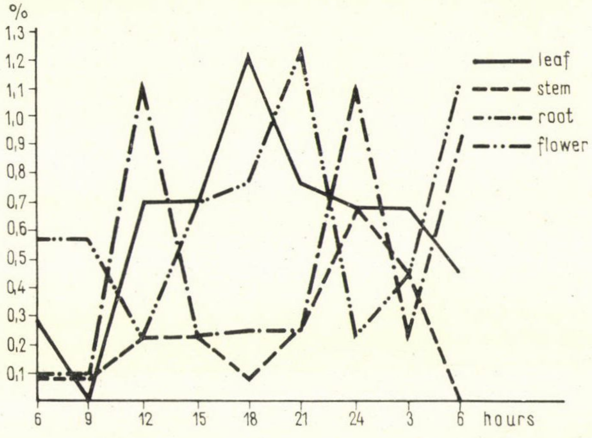

Fig. 15. 2nd exp. Daily changes of starch in Gamba grass

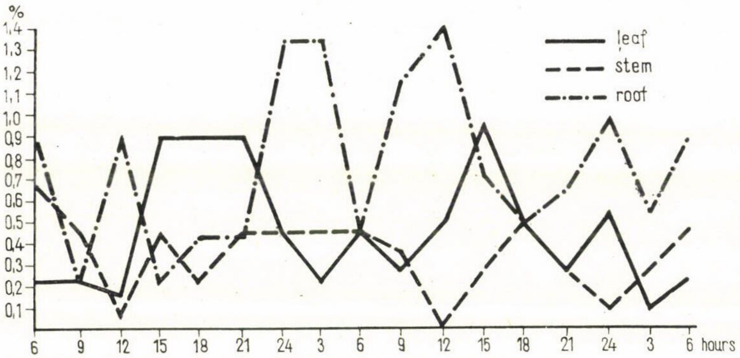

Fig. 16. **Changes of starch content in Gamba grass during the 48 hour experiment**

Discussion

The investigation of the daily changes revealed that there was no direct connection between the production of carbohydrates during photosynthesis and the concentration of free sugars in the plant. Evidently, the content of free sugars in the grass leaves is under the influence of complex regulating mechanisms. A further study of these regulating mechanisms is necessary (and is planned), before we can explain these changes in the case of Northern Gamba grass. However, it is possible to analyse some of the problems involved in more general terms. Glucose, fructose and their phosphorylated derivatives are among the early products of photosynthesis. They are readily interconvertible. Till recently, we thought that the Calvin cycle was the only pathway for carbon in photosynthesis and accordingly the ribulose-diphosphate was the only acceptor of carbondioxide in photosynthesis. Recent investigation on some tropical grasses like sugar cane, however, show that another compound, phospho-enol-pyruvic acid may also act as a carbondioxide acceptor. (This reaction was known for a long time, it was, however, not connected with photosynthesis). The relative importance of the two pathways is not yet known. We do not know if they act parallel, and how in that case their relative contribution to the total carbondioxide fixation is regulated. The sucrose is built up from glucose and fructose, and it can be decomposed to the same compounds, but these processes are not so simple as they seem to be. According to our present knowledge, the synthesis of sucrose takes place in two steps. In the first step sucrose-phosphate is synthesised from uridinediphospho-glucose (UDPG) and fructose-6-phosphate by sucrose phosphate synthetase. In the second step this is hydrolysed by a phosphatase to sucrose and to inorganic phosphate. The decomposition to sucrose might probably take place in two ways, by the so-called sucrose synthetase, which splits the sucrose to fructose and to UDPG, and by invertase to glucose and fructose. The UDPG is also a starting point for the starch synthesis, and might be one for cellulose. According to some opinions, guaniodiphosphate-glucose substitutes the UDPG in the cellulose synthesis of higher plants. For a time it was thought that the sucrose synthetase is really an enzyme for synthesis, as its name still indicates. There were many doubts about the role of invertase in plants, until we learnt it is under regulation in sugar cane, which means that it has to be involved with the sucrose metabolism.

Sucrose is not only involved in the metabolism of hexoses and polysaccharids, but it is the form in which the carbohydrates are translocated in the phloem. Its quantity at any given moment in a plant tissue derives not only from its synthesis and decomposition, but perhaps even more from its translocation for storage. These processes are all regulated and some details of the process have already been described.

In discussing the role of starch, we shall have to start from general conclusions. In cells the plastids are the cytoplasmatic organelles with the ability to make starch. Those cells which are not able to form starch under natural conditions can be induced by sugar solution to perform the process in higher plants. The monocotyledons are exceptions, because in their cells the starch synthetising enzymes are absent or inhibited, or the starch precursor content is too low. In such cases a sugar solution supply can induce starch production as pointed out in 1969 by Badenhuizen.

In cells for starch biosynthesis two systems are considered: 1. From glucose-1-phosphate long chains are built up by starch phosphorylase in which the glucose residues are linked together by α-1.4 bonds. The reaction is allowed to proceed in the presence of a primer or starter molecule with the same glucosidic bonds, and containing at least three glucose residues. 2. UDPG-α-glucan-glucosyl transferase transfers a glucose unit from UDPG to an oligosaccharide or a starch molecule.

In order to form starch, the presence of phosphorylase is considered the most important in higher plants. Several inhibitors of phosphorylase are known among which are inorganic salts, phosphates, iodine and β-amylase.

With grasses (e.g. *Cynodon*) amylase is the most likely inhibitor. The amylase activity can be suppressed by a small concentration of mercuric chloride, and the less sensitive phosphorylase will be able to induce the starch formation if the glucose-1-phosphate level is also sufficient.

The role of the ADPG or UDPG-glucan transferase is questionable as an individual enzyme in the starch synthesis, but it has an important function in the production of oligosaccharide from maltose, which could then act as primers for phosphorylase (BADENHUIZEN 1969).

We would like to point out, however, that the knowledge of metabolic pathways and their immediate regulation would not be enough to answer the simple question why the change of sugars in Gamba grass follows the pattern we described. Further complications arise from the fact that almost all of the physiological and biochemical parameters of higher plants show a diurnal change, which is called circadian rhythm (SWEENEY 1969). The circadian rhythms were at first recognized in the leaf movement of some tropical plants, but later, after the research of Bunning, it became evident that the same rhythm can be the basis of the photoperiodic reactions. Unfortunately, we do not yet know well the biochemical mechanism involved in the circadian rhythm. Two main theories have been postulated. According to the hypothesis of Bunning, the plants have a regular interval oscillation in some components of their metabolism, and, depending on the phase of this oscillation, they react differently to external controlling factors.

The other hypothesis, we may call the hourglass theory, which presupposes that the change of environment will set up a specific change in the compound and, depending on the quantity of this its reaction, may change. There are many reasons to believe that this key compound is a specific compound and may be the so-called phytochrome.

At the present moment it is not possible to decide which theory will be correct, but it is quite possible that a new theory will develop which will contain some elements of both.

From our point of view it might be enough to stress that the metabolic processes of higher plants are probably under the effect of a further super-regulation, which will need to be understood before one can proceed to analyse the behaviour of the soluble carbohydrates and starch that have been recorded in these observations.

Suffice it to say, that from the point of view of its agricultural value, the low content of soluble carbohydrate and starch observed in the leaves of Northern gamba grass may account for the poor quality of silage that has been produced from this species as recorded by MILLER—RAINS—THORPE (1963). In using this species for silage, it will be useful to incorporate a high-energy preservative like molasses or ground corn.

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THE COAGULATION ON HIGH FREQUENCY OF INCUBATED EGGS FOR PATHOLOGICAL INVESTIGATIONS

By

J. NAGY, A. PÁL

UNIVERSITY OF AGRICULTURAL SCIENCES, DEBRECEN

A method of fixation of the clinical symptoms of decayed eggs, which does not distort the feature of the disease, and does not inhibit the culture of pathogenes was experimented by high-frequency coagulation. According to our observations, this condition is brought about by the following physical properties. Goose eggs of average size after 8—10 days of incubation can be coagulated within 15 min. at an average in the coil of a HF oscillator. In the case of hen eggs of average size after 8—10 days of incubation 12 min. were necessary for coagulation with a smaller-power oscillator of convenient coil diameter. According to our experimental results, the anode current of oscillators shows the closest correlation with the density of the eggs. The results of the microbiological investigation are as follows. On mediums of potato-b and beer-agar, fungi belonging to *Aspergillus*, *Penicillium* and *Mucor* genus were cultivated of the samples obtained after coagulation on high frequency. On meat-agar and Klimert medium *E. coli. Streptococcus* and *Staphylococcus* colonies were cultivated after coagulation.

Introduction

Numerous embryo destructions taking place eruptively in connection with fowl incubation on the coops make it necessary to get to know the exact site of pathological alterations. It is of great importance not only in research work but in practice as well. The investigation, and thus the incubation is very difficult, even impossible because of their liquid aggregation.

The different layers of the eggs decayed in the period mentioned can be fixed by several methods of coagulation, but most of them alter, distort the feature of the disease, and make it impossible to note the condition of coagulation suitable for investigation during the treatment.

After the treatment on high frequency used by us the coagulated layers show a transition between a viscous and a stable condition, the feature of the disease is not altered significantly, the different components only show a slight deviation, and can be separated easily.

Our method can lead to positive examination results even in lack of clinical symptoms by investigating the relation between the weight of the different layers and the incubation percentage.

Coagulation on high frequency makes it possible to control the alteration of the conditions suitable for dissection. Due to the change of anode-current the coagulation condition suitable for morphological studies does not kill the microorganisms, so the bacteriological and mycological investigation of the separated layers is also possible.

Material and method

We used two types of HF oscillators of different output power for the coagulation of incubated eggs. In both cases we put the eggs in the coil of the oscillators one by one.

The oscillator of low power (with Tungsram OT 100 tube) with self-led capacitive feedback was used by us in the case of hen eggs. This on a high frequency could yield an output power of $P_{max} = 100$ watts at $f = 34$ Mc/s, if the anode potential $U_a = 1300$ volts. In the case of both oscillators we could infer the output power from the anode current.

The hen eggs put into the coil with 8 worms ($l = 6$ cm, $\varnothing = 5$ cm) showed a dissipation of a few watts at the beginning, and of 14—25 watts by the progressing of the coagulation.

We devised a convenient oscillator for the coagulation of incubated goose eggs. Their coil was made of copper with 14 worms ($l = 10$ cm, $\varnothing = 7$ cm). This oscillator could yield a useful HF power of $P_{max} = 250$ watts on $f = 26$ Mc/s frequency at an anode voltage of $U_a = 2400$ volts (Tungsram OQQ 151/3000 transmitting tube).

The goose eggs put into the coil showed a dissipation effect of a few watts at the beginning, and took off 37—45 watts during the treatment of 15 minutes. In both cases we were very careful that the eggs should not come in contact with the worms of the coil to avoid the electrical arc, and in addition we used an external air cooling.

The coagulated eggs were dissected in a sterile box, their opening was made in three stages worked out by the authors. The colour or morphological alternations were observed macroscopically, samples were taken with a glowing platinum stick, and laid on the medium in a zig-zag form. Meat-agar and Klimert medium were used for the bacterium culture, and potato-b and beer-agar for the fungi culture.

Results

The results of a series carried out on nearly 2000 individuals show that in the case of goose eggs an average anode current of 50—54 mA was necessary to obtain a coagulation condition suitable for dissection. In the case of egg weight ranging between 130—180 g this was brought about within 15 min. (Fig. 2a) and during this period the HF power-dissipation was between 37—45 W referring to the weight category mentioned above.

Searching for the factor on which the size of the anode current mostly depends, according to the results of a correlative investigation, it was found that correlation was the closest between the density of goose eggs and the ampere: +0.989, whereas it was +0.959 between the weight and ampere, and the least: +0.804 was between the volume and ampere. In case of goose eggs, the physical characteristics belonging to the different weight categories can be seen in the *a*-columns of Table 1.

The causes of mortality are: mycosical, 10.09%, bacteriological, 23.34%, sterile, 33.50%, and other causes, 32.07%. The latter was represented in many

Table 1

The physical properties of coagulated eggs (goose a, hen b) for dissection

Weight of eggs, g		Volume, cm³		Density, g/cm³		Average anode current, mA		Dissipated power, watt	
						15 min	12 min		
a	b	a	b	a	b	a	b	a	b
130—140	40—45	134	43	1.007	0.988	50.6	61.75	37.44	14.1
140—150	45—50	145	46	1.000	1.034	50.4	66.75	36.96	20.1
150—160	50—55	148	48	1.047	1.093	51.4	69.00	39.36	22.8
160—170	55—60	150	50	1.100	1.150	53.0	76.75	43.20	23.5
170—180	60—65	155	52	1.129	1.202	54.0	86.00	45.60	25.5

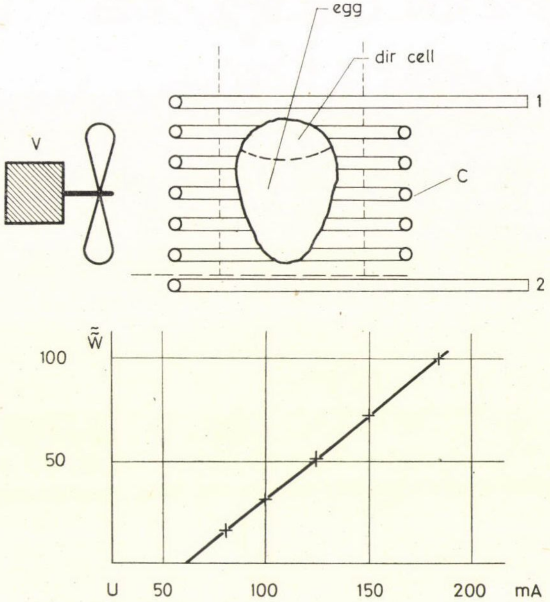

Fig. 1. The HF output of the low-power oscillator as a function of the anode current (C = HF-coil, V = ventillator)

cases by a small volume of dense albuminous layer, and could be inferred decidedly from genetical causes (about 1% uncoagulated).

After HF coagulation on a medium of potato-b and beer-agar, we succeeded in culturing species belonging to *Aspergillus* genus out of the shell-membrane and embryo, to *Penicillium* genus out of the shell-membrane, to Mucor genus out of the different layers of yolk depending on the severity of infection.

On a medium of meat-agar and Klimert *E. coli*, *Streptococcus* were cultured after treatment on high frequency, species belonging to other genuses were also produced, but this could not be established without doubt, although it was sure, they were gram- negative. In case of *E. coli*, the most massive culture was produced from samples taken of granuloma on the shell-membrane, less massive culture was produced from the embryo, and the samples taken from different layers of the yolk. We did not succeed in culturing anything from chalaza.

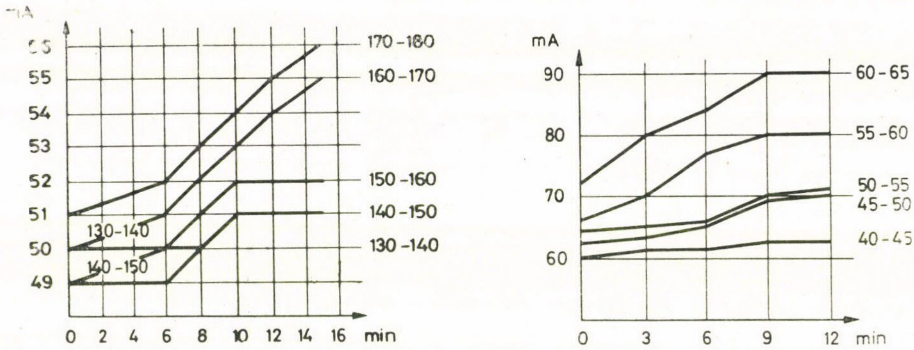

Fig. 2. The relationship between the anode current and time according to different weight categories in case of goose (*a*) and of hen (*b*) eggs

According to our measurements carried out on nearly 400 hen eggs, an average anode current of 61—86 mA was necessary in case of egg weights ranging between 40—65 g to obtain a coagulation condition needed for dissection. It was brought about within 12 min. (Fig. 2b), and during this period the output power referring to the weight category mentioned above was between 14—25.5 W.

As in the case of goose eggs, it could be established here, too, that correlation was the closest between the density of eggs and the anode current: +0.989, whereas it was +0.986 between the current and the weight of eggs, and finally it was the least between volume and current +0.961.

The percentual division of mortality causes as well as the microorganisms, genuses cultured from different parts of the hen eggs, and the media are nearly the same as those of the goose.

Discussion

It is well-known that after a long period heat coagulation may take place even at 57° C, but after a short period only opalization can be observed at 58°C in case of hen eggs (Romanoff 1949). In case of wild birds like pigeon, robin, starling, even 80—90°C is needed for coagulation (Romanoff 1949). In our study the internal heating effect of high frequency is responsible mostly for precipitating coagulation.

There may be even other ways of coagulation but these are not good for dissection. BANCROFT—RUTZLER (1931) showed that egg albumin can be coagulated by different drugs. There may even be other coagulating agents, e.g. mechanical shaking (WU—LING 1927), pressure of about 5000 atm. or higher without increasing temperature (ROMANOFF 1949, GRANT *et al.* 1941).

The rise of HF dissipation is possibly determined by the density of ion concentration. It was observed that the power dissipation of distilled water put into the coil of our oscillator is less than that of the same volume of tap water. In the case of greater ion density coagulation occurs at a higher anode current.

With our experimental method one can differentiate even in lack of traditional pathological symptoms, as the egg components can be investigated and measured, for example, the yolk on the size of which the bodily strength and vitality of the embryo and chicken depends or the dense outer albumin layer, the thickness of which depends on the season and the storage of eggs (MOLNÁR 1966). According to KISS (1962), there is a relationship between the per cent of hatching and the quantity of the dense albumin layer.

The coagulation on high frequency of the eggs incubated for 8—10 days, as it appears from what was mentioned above, is brought about at 55°C (measured immediately after the HF coagulation on the outer surface of the eggs) and in comparison in a relatively very short time (12—15 min). The cause of it is as follows. In case of the traditional ways of coagulation the heat conduction takes place incompletely by way of transmission from layer to layer, and the different layers may be coagulated in one another. Whereas, in the case of our method, on high frequency described earlier, heating takes place simultaneously in each layer. This process makes the separation of each layer possible. It is assumed that the movement in a space of high frequency of the ions found among the protein molecules may help coagulation locally as well. The coagulation process takes place simultaneously in the whole volume, and in the heat transmission the conduction of the heat has a little, even negligible role. The practical importance of coagulation on a relatively low temperature is that pathogens closed in the eggs could be cultured even after coagulation.

Acknowledgements

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SEASONAL CHANGES IN THE K AND Ca CONTENTS OF TERRICOLOUS XEROPHYTON LICHEN SPECIES AND THEIR SOILS

By

E. Kovács-Láng, K. Verseghy

DEPARTMENT OF PLANT TAXONOMY AND ECOLOGY
OF THE EÖTVÖS LORÁND UNIVERSITY, BUDAPEST;
BOTANICAL COLLECTION OF THE MUSEUM OF NATURAL SCIENCES, BUDAPEST

The seasonal changes in the K and Ca contents of four xerophyton-lichen species (*Cladonia magyarica, Cladonia furcata, Cladonia convoluta* and *Parmelia pokornyi*) from *Brometum tectorum secaletosum* and *Festucetum vaginatae* grassland communities of sandy soils were studied over two years. The Ca content of lichens is much higher, the turnover of Ca quicker and the time of its replacement considerably shorter compared to the potassium. Taking the phytomass of the lichens, the amount of Ca contained in them and the rate of Ca turnover into consideration, it can be established that their role in the calcium turnover of the plant communities studied is more important than that of the Gramineae species associated with them. In the potassium turnover of the plant communities studied the role of lichens is much less important.

Introduction

In the course of investigations carried on for years in the framework of the IBP–PT we succeeded in forming a notion of the phytomass production in the vegetation period of lichen species in an annual *(Brometum tectorum secaletosum)* sandy grassland community characteristic of the calcareous sandy areas of the Great Hungarian Plain, and in a perennial open grassland community of sandy soil *(Festucetum vaginatae danubiale)*, respectively (Verseghy–Láng 1971); of seasonal changes in their chlorophyll contents (Verseghy 1972) and of their water regime (Verseghy 1971).

The present study is aimed at determining the role lichens play in the mineral turnover of these plant communities. As a first step, the seasonal changes in the quantities of two ions — Ca^{++} and K^{+} — important at the site, were examined in four lichen species and their soils. We did not, however, want to find out in what compounds and in which parts of the lichen thallus the two cations examined occur.

The mineral turnover of lichens was first dealt with by Salomon (1914) who made rough estimates of the ion store of the algal and fungal components of lichens.

Certain lichen species excel in their high capacity for metal accumulation — as pointed out by some authors (cf. Hale 1967). A mineral content higher than their substrates can be observed in lichen species living on the barks of trees too (HALE 1967). According to BAZILEVICS—RODIN (1971), a high mineral content occasionally amounting to as much as 10 per cent of the dry-matter content is a characteristic feature of xerotherm lichen species in the Russian steppe zone. The Si and Ca contents are especially high compared to a strikingly low N content.

Material and method

Our investigations were made in the Great Hungarian Plain, on a calcareous sandy area of the Danube—Tisza Mid-region, with samples taken every month between August 1970 and April 1972. From selected stands of annual *(Brometum tectorum secaletosum)* and perennial open *(Festucetum vaginatae danubiale)* sandy grassland communities two varieties (var. *palamaea*) Ach. (Nyl.), var. *subrangiformis* (Sandst.) Abb. of *Cladonia furcata* a mixed material occurring in masses in the communities studied, and the species *Cladonia magyarica* Vain., *Cl. convoluta* Lam. and *Parmelia pokornyi* (Körb.) Szat. were collected. From the carefully cleaned and sorted out material 3 g air-dry samples (in 2—3 replications per species) were analysed. The plant material was digested in a mixture of concentrated H_2SO_4 and H_2O_2 with Se catalizer used after Kjeldahl's method, and K^+ and Ca^{++} were determined by means of flame photometry. The solution, before being subjected to flame photometry, was freed from sulphates on a Varion AD-type anion-exchanging resin column. The measurement results are given in mg% related to the dry matter content of the lichens.

The soil samples were taken every month from a 1 cm layer under the lichens, simultaneously with the plant material. From the air-dry soil an aqueous solution of soil + distilled water of 1 : 5 ratio was prepared which — after being shaken, sedimented and filtered — was subjected to flame photometry. The turnover rate (K) and turnover time (1/K) were determined after ROBERTSON (1957) and PRÉCSÉNYI (1971) on the basis of the following correlation; $K = L/x$, where L = the annual increment of matter (e.g. Ca, K), x = the maximum value of matter. The statistical analyses were made after SVÁB (1967).

Results

Seasonal changes in the Ca content. In the grassland communities examined the bulk of the phytomass is made out of organic matter produced by lichens and mosses (VERSEGHY—LÁNG 1971). Flowering plants are represented in the highest proportion by grasses *(Festuca vaginata, Koeleria cristata, Bromus tectorum, Secale silvestre)*. It is known (TÖLGYESI 1969) that Gramineae are characterized by a low Ca content, therefore in these grasslands lichens play a more important role in the turnover of Ca.

The Ca content of the Hungarian xerotherm lichen species examined is 1000—3000 mg% higher than that of the common grasses of Hungary — Ca content related to the dry matter content is 150—240 mg% in *Festuca pallens*, 250 mg% in *Koeleria cristata*, 340 mg% in *Bromus tectorum*, 100—180 mg% in *Festuca pseudovina* and 350 mg% in *Festuca sulcata* (KOVÁCS-LÁNG 1966, TÖLGYESI 1969). The Ca content of lichens living in the annual grassland of sandy soil is higher (1000—3000 mg%) — in the case of the same species — than in the perennial *Festucetum vaginatae* (1000—2000 mg%).

The Ca content of lichens shows seasonal fluctuations. Thus, e.g., Ca content in *Cladonia magyarica* occurring in masses in the *Festucetum vaginatae* plant community shows a maximum in autumn and winter (3300, 2600 mg%), while in spring and summer it is uniformly lower (1000 – 1200 mg%; Fig. 1). The same species can be found in *Brometum* in a lower quantity, the maximum of its Ca content appears at a different time, 1 – 2 months earlier (3600, 2300 mg%).

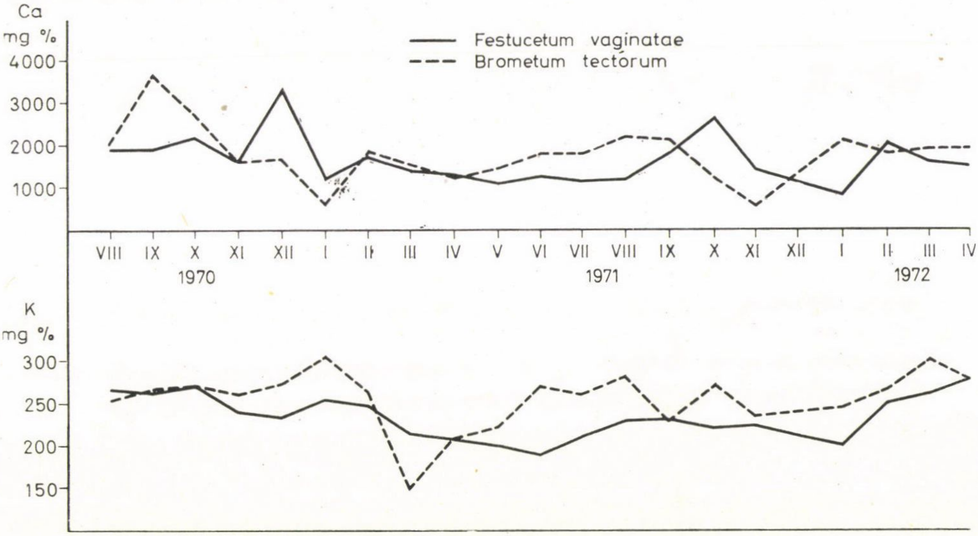

Fig. 1. Seasonal changes in the K and Ca content of *Cladonia magyarica* in the plant communities studied

Cl. furcata (var. *palamaea* and var. *subrangiformis*) is the dominant lichen species of *Brometum*, though occurring in considerable quantities in the *Festucetum vaginatae* community too. The seasonal changes of its Ca content are similar in the two plant communities, lower in summer (1000 – 1100 mg%) and of an increasing tendency in autumn. The Ca content is high in winter too. It is maximum in September (Fig. 2); 2900 mg% in *Brometum* and 2300 mg% in *Festucetum vaginatae*.

Cl. convoluta forms a smaller mass in both communities than either of the two former species. The seasonal changes in its Ca content show by and large a similar picture as seen in the former species; its lower summer value (1000 mg%) is followed by autumn- and winter maxima (Fig. 3); 2700 mg% in *Brometum*, 3400 mg% in *Festucetum vaginatae*.

The amount of *Parmelia pokornyi* is only significant in the *Festucetum vaginatae*. Its average Ca content is higher than that in the other species examined; the maximum value is in December (Fig. 4) (4200 mg%), the minimum in May (1100 mg%).

Seasonal changes in the potassium content. The K content of lichens is much lower than their Ca content — 2—300 mg% on an average — and its seasonal fluctuation is also lower. The K content of flowering plants is many times higher than that. Thus the role of lichens in the turnover of potassium within the community is much less important than in the case of Ca. The K content of lichen species is of similar value in both communities.

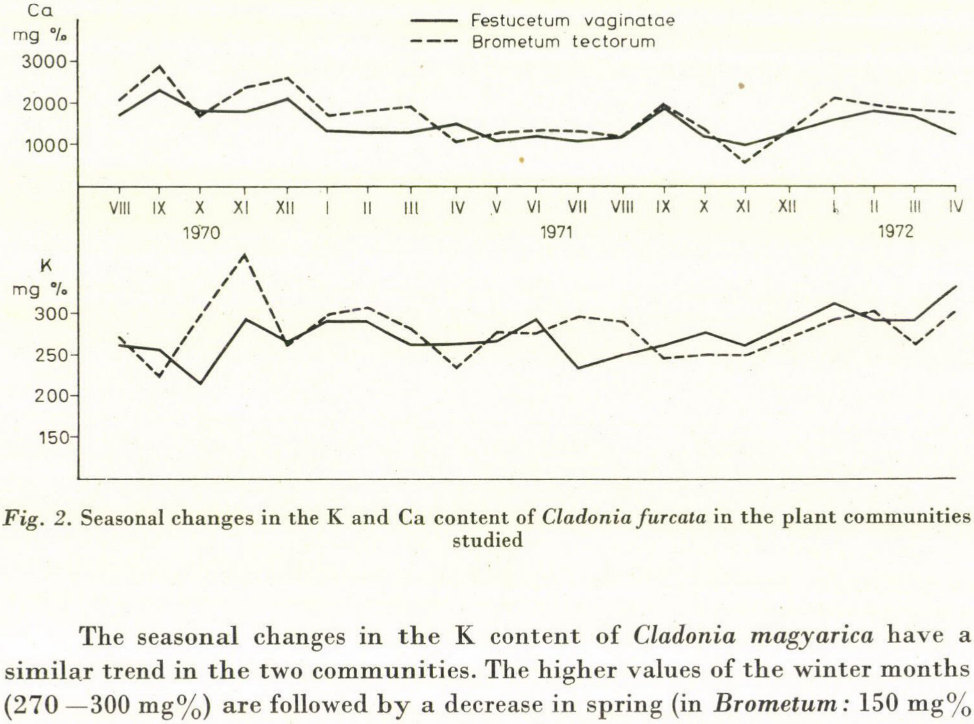

Fig. 2. Seasonal changes in the K and Ca content of *Cladonia furcata* in the plant communities studied

The seasonal changes in the K content of *Cladonia magyarica* have a similar trend in the two communities. The higher values of the winter months (270—300 mg%) are followed by a decrease in spring (in *Brometum:* 150 mg% in March, in *Festucetum vaginatae* 190 mg% in May; Fig. 1). The maxima of the K and Ca contents do not appear at exactly the same time.

The trend of K content in *Cl. furcata* is also similar in the two communities, only in the *Brometum* the fluctuations are higher. The tendency is similar to that of the first species, however, the decrease in spring is less expressed, and the summer values are not so low either (Fig. 2), 230 mg% in *Brometum,* 250 mg% in *Festucetum vaginatae.*

The maximum and minimum values of K and Ca contents do not coincide exactly, the maxima of K precede those of Ca.

In winter *Cl. convoluta* reaches a relatively uniform and not too high value in both communities (250 mg%) which — after an expressed spring (May—June) minimum (180 mg%) and slow summer increase (240 mg%) —

is followed by a second decrease in October (200 mg%), until — finally — the winter level is gradually attained (Fig. 3). Changes in the K content occur parallel to those of the Ca content; maxima and minima appear at about the same time.

The K content of *Parmelia pokornyi* ranges between similar value limits as in the former three species. Its seasonal changes show the following trends:

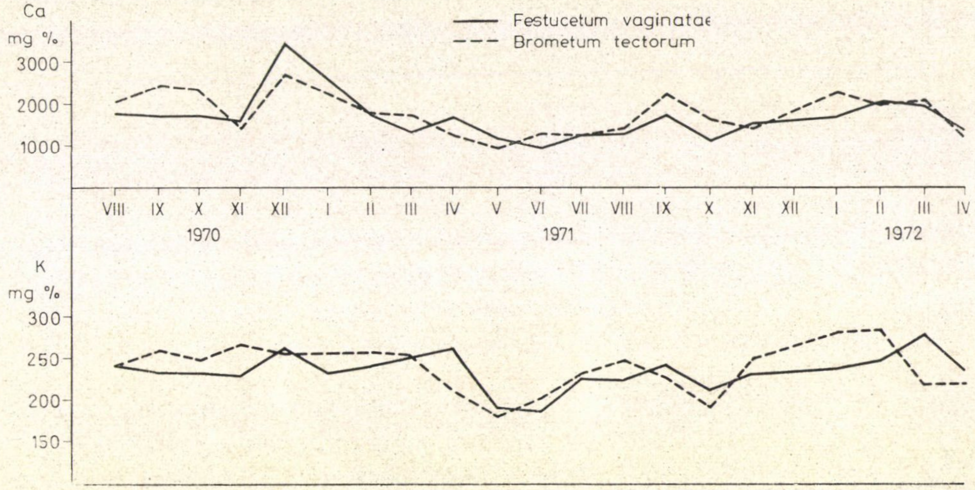

Fig. 3. **Seasonal changes in the K and Ca content of *Cladonia convoluta* in the plant communities studied**

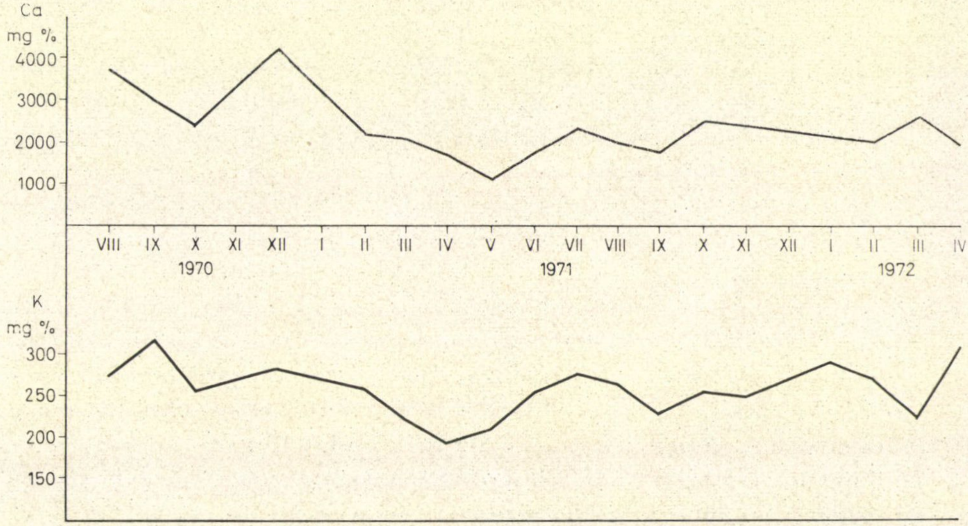

Fig. 4. **Seasonal changes in the K and Ca content of *Parmelia pokornyi* in *Festucetum vaginatae***

it is lower (200 — 250 mg%) in spring and autumn, and higher (270 — 300 mg%) in summer and winter (Fig. 4).

When comparing the potassium and calcium contents of the different species we can see that the trend of the seasonal changes and the quantitative values are similar, but *Parmelia pokornyi* has a higher Ca content than the other species.

When the potassium and calcium contents of the same lichen species are compared in the two plant communities, an expressed difference is only found in the Ca content of *Cl. furcata* which is higher in the *Brometum*.

The turnover rate of potassium and calcium contents. The turnover of phytomass in Hungarian plant communities *(Artemisio-Festucetum pseudovinae, Peucedano-Galatelletum punctati)* was studied by PRÉCSÉNYI (1971). Data on the xerotherm lichen species of Hungary are presented by SIMON — KOVÁCS-LÁNG (1972).

An essential difference is shown in the dynamics of the two minerals studied by us. The values of seasonal changes suggest that in the period examined the turnover of calcium is much quicker in the lichens than that of potassium. As to the weather conditions, the two years of the period of investigation were highly different.

It was the extremely dry summer of the second year (1971) that had an especially unfavourable effect on the vegetation, as felt in a considerable decrease of lichen production too.

In the two years of the period of investigation the calcium turnover of lichens did not show essential differences; neither the rate nor the time of turnover changed significantly in the successive years. In the annual grassland of sandsoil the calcium turnover was somewhat quicker, the turnover time shorter than in the perennial grassland (Table 1).

The potassium turnover showed different trends. The behaviour of the same lichen species was not identical in the two plant communities. The species examined displayed a more intensive potassium turnover — their turnover time was shorter in both years — in the *Brometum tectorum* community than in the perennial grassland. The potassium turnover of *Cladonia furcata* and *Parmelia pokornyi* slowed down in the second, unfavourable year in both communities, while in *Cladonia convoluta* accelerated. In the unfavourable year the potassium turnover of *Cladonia magyarica* became quicker in the *Festucetum vaginatae* community, and slower in the annual grassland.

From our results we have drawn the conclusion that the mineral turnover of lichens cannot be separated from that of the flowering plants occurring in the same plant community. In the annual grassland of sandsoil where the organic and mineral matters of flowering plants are almost completely renewed in the course of a year, the turnover is quicker in the lichens too. That is why the turnover time of their mineral contents — first of all potassium —

Table 1

Values of turnover rate (%) and turnover time (year) of Ca and K in the lichen species of plant communities studied

	August 1970–April 1971				April 1971–April 1972			
Festucetum vaginatae	Ca		K		Ca		K	
	%	year	%	year	%	year	%	year
Cladonia magyarica	62	1.61	23	4.35	68	1.47	30	3.33
Cladonia furcata	42	2.38	20	5.0	46	2.17	14	7.14
Cladonia convoluta	61	1.63	12	8.33	53	1.88	32	3.12
Parmelia pokornyi	59	1.70	39	2.56	56	1.78	31	3.22
Brometum tectorum								
Cladonia magyarica	82	1.22	51	1.96	75	1.33	27	3.7
Cladonia furcata	61	1.63	38	2.63	73	1.37	18	5.55
Cladonia convoluta	56	1.78	21	4.76	57	1.75	36	2.77

becomes shorter. The phytomass of the flowering plants forming the perennial grassland is but partly replaced in a year, the turnover of matters is slower. This slower rhythm is reflected in the lichen species too.

Changes in the water- soluble ion content of the soil. When analysing the potassium and calcium contents of the 1 cm soil layer under the lichens of the *Festucetum vaginatae* community, we find that they are in a positive correlation with one another and with the pH of the soil. The correlation coefficient between the K and Ca content is 0.69 ($P = 0.1\%$).

The water- soluble potassium and calcium content of the soil is low and shows considerable seasonal fluctuations. The amount of calcium is 0.5 –1.5 mg%, that of potassium 0.1 –0.2 mg% as related to an air-dry soil. The highest values appear in winter, the lowest ones in the rainy spring months when, with the beginning of the vegetation period, the more intensive mineral uptake of the flowering plants also contributes to a decrease in the ion content of the soil. In the drier summer period the amount of minerals increases again. The minima at the end of winter may originate from a wash-out by the melting snow (Fig. 5). The seasonal changes in the pH values correspond to the changes of cations.

No correlation could be demonstrated between the water-soluble potassium and calcium contents in the lichens and those in the 1 cm soil layer under them. It is interesting, however, that both in the soil and in the lichens the mineral content is higher in winter than in summer, and an expressed increase can be observed in spring. Actual correlation might be found with a refined methodology.

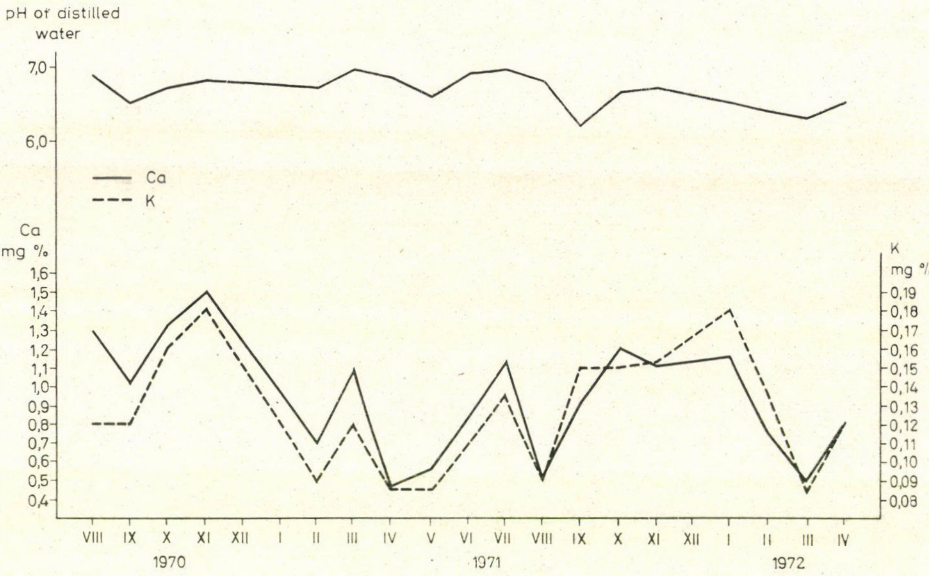

Fig. 5. Water-soluble Ca and K content and the trend of pH in the soil of *Festucetum vaginatae*, in the upper 1 cm layer under the lichens

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GENETIC INVESTIGATION IN AEGILOPS×TRITICUM COMBINATIONS. I

AE. TRIUNCIALIS×TRITICUM

By

K. K. Ghoshal, A. Belea

DEPARTMENT OF GENETICS AND PLANT BREEDING, FACULTY OF AGRICULTURE,
UNIVERSITY OF KALYANI, KALYANI, W. BENGAL;
INSTITUTE OF GENETICS CENTRE FOR BIOLOGICAL RESEARCH, HUNGARIAN ACADEMY OF SCIENCES,
SZEGED

Although the genome relationship between *Aegilops* and *Triticum* and the evolution of different species under them have been thoroughly worked out, they do not always behave in accordance with their assigned genome formulae. Of late, there has been considerable rethinking about the affinity between these two genera. The present investigation was a part of an elaborate programme undertaken to collect more data on the crossability, fertility of hybrids and on their cytological and morphological characteristics. *Ae. triuncialis* was found to cross differentially with tetraploid and hexaploid *Triticum*. The percentage of seed setting and sprouting of hybrid seed was strikingly high in combinations with hexaploid *Triticum*. The fertility of F_1 hybrids was not so high. Cytological examination showed pairing of chromosomes in the meiosis of F_1 hybrids. Morphologically, the hybrids were mostly intermediate between the parents. It seems that the genomic formula assigned to *Ae. triuncialis* needs revision to explain the high seed setting and sprouting of hybrid seeds and meiotic pairing observed in combinations between *Ae. triuncialis* and tetraploid and hexaploid species of *Triticum*

Introduction

Following the pioneer and all-embracing genome analytical work of the Kihara school, the origin and evolution of different genomes of *Aegilops* were considered to be a closed issue. The genome formulae assigned to different diploid, tetraploid, and hexaploid species of *Aegilops* by Kihara and his collaborators (Kihara 1954, 1963) had been accepted almost universally. However, these symbols were not always the result of concrete and detailed analysis (Chennaveeraiah 1960). It has been found that in many interspecific and intergeneric crosses, the different species of *Aegilops* do not always behave in complete agreement with their genome symbols. Recently, Kihara himself felt the necessity of a revision of these symbols, since new and useful data had accumulated (Kihara—Tanaka 1970).

With this background, we tried to collect new data on the crossability of different species of *Aegilops* and *Triticum* and also on the fertility and cytological and morphological behaviour of the resultant hybrids.

Material and method

Seeds of *Ae. triuncialis* used in our experiments were collected from the Soviet Union. The tetraploid and hexaploid species and cultivars of *Triticum* were obtained from the collection of the Agricultural Research Institute, Martonvásár. Experiments were conducted at the experimental garden of the institute. Plants were raised with a spacing of 30 × 20 cm.

In all our crosses, *Ae. triuncialis* was used as the female parent, since past experiences show that better seed-setting is obtained in *Aegilops-Triticum* crosses if the *Aegilops* species acts as the female parent.

Special effort was made to synchronise the flowering time of the two parents by sowing on different dates.

Hybrid seeds obtained from the crosses were first sown in small pots and in case of successful sprouting, the young seedlings were transferred to the experimental garden. After sprouting, critical observations were made in the field for hybrid lethality and survival. Morphological observations were made in the field on all the hybrid plants and their parents.

After the harvest, data on different quantitative characters, like height of plants, number of tillers, length of main and secondary ears, spikelets per ear, number of seeds in the main and secondary ears were collected in the laboratory.

Cytological examinations were performed at meiotic level with leucobasic fuchsin following the technique suggested by the Plant Breeding Institute, Cambridge. For studying the pollen grain stainability, fully mature anthers were fixed and stored in Carnoy's solution. At the time of the observation the pollen grains were dusted in a drop of 1 per cent acetocarmine and then mounted. Generally full, round and darkly stained grains were treated as stained pollens.

Results

Table 1 gives an account of the hybridization attempts between *Ae. triuncalis* and different tetraploid species of *Triticum* performed in 1968 and 69. Though seed-setting was observed in most of the cases, the hybrid seeds were mostly sterile and only in the case of *Ae. triuncialis* × *T. turgidum*, was 14.28 per cent sprouting recorded. The crossing attempts between *Ae. triuncialis* and different cultivars of hexaploid *Triticum aestivum* gave a different picture. In almost all the cases seed-setting was unexpectedly high (Table 2). However, considerable variation was observed among the cultivars in terms of ability to set seeds. In 1969, the cultivar San Marino produced the highest percentage

Table 1

Records of hybridization between Ae. triuncialis and tetraploid Triticum

Combination	Year	No. of flowers crossed	No. of seeds obtained	Percentage of seed-setting	Percentage of sprouting
Ae. triuncialis × *T. dicoccoides*	1968	18	1	5.56	0
Ae. triuncialis × *T. turgidum*	1968	61	6	9.84	0
Ae. triuncialis × *T. turgidum*	1969	140	7	5.00	20.00
Ae. triuncialis × *T. durum*	1969	42	0	0	0
Ae. triuncialis × *T. carthlicum*	1969	84	1	1.19	0
Ae. triuncialis × *T. timopheevi*	1969	56	0	0	0

Table 2

Records of hybridization between Ae. triuncialis and hexaploid Triticum

Combination	Year	No. of flowers crossed	No. of seeds obtained	Percentage of seed-setting	Percentage of sprouting
Ae. triuncialis × *T. aest.* (Bezostaya-1)	1969	184	26	14.13	26.92
Ae. triuncialis × *T. aest.* (Rannaya-12)	1969	42	4	9.53	75.00
Ae. triuncialis × *T. aest.* (Champel)	1969	62	6	9.68	16.67
Ae. triuncialis × *T. aest.* (San Pastore)	1969	66	6	9.06	83.33
Ae. triuncialis × *T. aest.* (Noria 10)	1969	30	5	16.67	60.00
Ae. triuncialis × *T. aest.* (Norin 70)	1969	30	3	10.00	100.00
Ae. triuncialis × *T. aest.* (Norin 16)	1969	32	4	12.50	30.00
Ae. triuncialis × *T. aest.* (Ranka III)	1970	48	4	8.33	0
Ae. triuncialis × *T. aest.* (Avrora)	1970	94	7	7.44	0
Ae. triuncialis × *T. aest.* (San Marino)	1969	24	5	20.83	100.00
Ae. triuncialis × *T. aest.* (Produttore)	1969	18	2	11.11	100.00
Ae. triuncialis × *T. aest.* (Kavkaz)	1970	48	8	16.67	0
Ae. triuncialis × *T. aest.* (F-293)	1969	158	22	13.93	40.91
Ae. triuncialis × *T. spelta* (Flaks. 6)	1968	134	1	0.75	0

of seed-setting, among the F_1 combinations. In 1970 the corresponding position was taken by Kavkaz, Ranka III and Aurora. Sprouting of hybrid seeds was surprisingly high and in certain cases even 100-percent sprouting was recorded (Table 2). *T. spelta*, the other hexaploid species tried in this experiment, gave very low seed setting.

Stainability of pollen grains and production of seed in the hybrids. Table 3 gives an idea about the pollen-grain stainability in the hybrids. The F_1 plant of *Ae. triuncialis* × *T. turgidum* gave quite a high percentage of stained pollen. This was not so high in the case of combinations with hexaploid *T. aestivum* cultivars.

In contrast to the production of stained pollen grains in a high percentage, the F_1 hybrid of *Ae. triuncialis* × *T. turgidum* yielded very few seeds. An idea about the seed production in F_1 hybrids can be obtained from Table 3. Among the F_1 combinations between *Ae. triuncialis* and *T. aestivum*, the one with Norin 10 gave the best seed yield in terms of seed/plant. In case of *Ae. triuncialis* × *T. spelta* the F_1 plants were highly sterile.

Cytological observation. In the F_1 of *Ae. triuncialis* × *T. turgidum* there was almost no pairing in meiosis. Out of the 16 pmc examined, only one showed a single bivalent in the M_1 of reduction division (Table 4). In the F_1 combinations of *Ae. triuncialis* with different cultivars of hexaploid *T. aestivum* var.

Table 3

Percentage of stained pollen and seed production in the F_1 hybrids

Combinations	Percentage of stained pollen grains	Seed/plant	Seed/ear
Ae. triuncialis × *T. turgidum*	23.69	1.00	0.03
Ae. triuncialis × *T. aest.* (Bezostaya-1)	1.95	1.50	0.08
Ae. triuncialis × *T. aest.* (Rannaya-12)	1.79	3.00	0.18
Ae. triuncialis × *T. aest.* (Norin 10)	2.64	3.67	0.12
Ae. triuncialis × *T. aest.* (Norin 70)	1.68	1.67	0.07
Ae. triuncialis × *T. aest.* (Norin 16)	—	2.00	0.22
Ae. triuncialis × *T. aest.* (San Marino)	—	1.60	0.06
Ae. triuncialis × *T. aest.* (Produttore)	—	3.00	0.12
Ae. triuncialis × *T. aest.* (F-293)	6.22	0.33	0.02
Ae. triuncialis × *T. spelta*	0.00	0.00	0.00

Bezostaya 1 and *Ae. triuncialis* × *T. aestivum* var. Norin 70, the most frequently occurring M_1 configuration was 2" + 31' (57.14 and 38 per cent respectively), though the maximum number of bivalents observed was 4 in both cases. In the case of *Ae. triuncialis* × *T. aestivum* var. F-293, 34 per cent pmc showed 3" + 29'. This was followed by 2" + 31' (32 per cent). Here the maximum number of bivalents observed in one pmc was 6. In one pmc a trivalent was observed (Table 4).

Morphlogy of F_1 hybrids. The F_1 hybrids were phenotypically intermediate between the two parents in most of the morphological characters. The ears showed a distinct influence of the *Aegilops* parent. In *Ae. triuncialis* × *T. aestivum* var. F-293 the ears were brown coloured with narrow glumes and short awns. Glume teeth were medium, coarse and pointed. The ears of *Ae. triuncialis* × *T. aestivum* var. Bezostaya-1 F_1 were also brown coloured with short awns. Glumes were medium, hairy, narrow, slightly convex having wide, pointed teeth.

The plant height was intermediate between that of the parents in the majority of the hybrids. But in the case of hybrids involving the cultivars San Pastore, Norin 10, Norin 70 and Produttore, the F_1 plants were taller than both the parents. The length of the main ear showed variable expression. In *Ae. triuncialis* × *T. turgidum* the F_1 had ears shorter than that of both the parents. The combinations of *Ae. triuncialis* with cultivars of *T. aestivum* mostly showed heterotic effect in this respect. In the case of Bezostaya-1, San Marino and F-293 the ear length was intermediate between that of the two parents. In the rest of the cases it was more in the F_1. However, the number of spikelets /ear was almost always intermediate between that of the two parents.

Table 4

Metaphase configurations of F_1 hybrids

Combination	Total no. of metaphase cell observed	Nature of the configuration	Frequency (No. of PMC)
Ae. triuncialis × *T. turgidum*	16	28'	15
		1"+13'	1
Ae. triuncialis × Bezostaya-1	84	35'	28
		1"+33'	1
		2"+31'	48
		33'	2
		3"+29'	4
		4"+27'	1
Ae. triuncialis × Norin 10	50	35'	6
		1"+33'	12
		2"+31'	19
		3"+29'	6
		4"+27'	7
Ae. triuncialis × F-293	100	1"+33'	2
		2"+31'	32
		3"+29'	34
		4"+27'	21
		5"+25'	9
		6"+23'	1
		1'''+4"+24'	1

Discussion

Early cytological examinations showed that tetraploid *Ae. triuncialis* contained the chromosome complement of *Ae. umbellulata* and *Ae. caudata*. This observation was strengthened by Sorokina (CHENNAVEERAIAH 1960) who synthesized a hybrid between these two species. Later on, KIHARA—KONDO (1943) successfully synthesized *Ae. triuncialis* by amphiploidizing the hybrid of *Ae. caudata* × *Ae. umbellulata* and subsequently the genomic formula C^UC was assigned to *Ae. triuncialis*. Since, according to the formulae given by KIHARA (1963), neither tetraploid nor hexaploid *Triticum* contains any of the two genomes of *Ae. triuncialis*, any crossing attempt between *Ae. triuncialis* and tetraploid or hexaploid *Triticum* is supposed to be abortive. CHENNAVEERAIAH (1960) called *Ae. triuncialis* a highly complex species. In his karyotypic observation on *Ae. triuncialis* ssp. *persica* and *Ae. triuncialis* ssp. *brachyathera* he found a set of chromosomes corresponding to the C^U genome. The second set of chromosomes was different from the C genome of *Ae. caudata* in both the cases and there was even a difference between these two sets of the two subspecies. In the absence of karyotypic examination of all the taxa belonging to this species complex Chennaveeraiah accepted the presence of both the C and C^U genomes in the *Ae. triuncialis* complex, but he was of the opinion that "in some either the C genome has undergone significant modification or there must be some foreign genome involved instead of the C genome". The present study strengthens this view by supplying crossing and fertility data. Our crossing experiments with tetraploid and hexaploid wheat, on the other hand, show quite encouraging results. Seed setting was observed in most of the crosses involving tetraploid wheat and in all the cases where hexaploid wheat was the male parent. The germination of hybrid seed was poor in the former instance but in the cases of crosses with different cultivars of *T. aestivum*, the germination was surprisingly high. However, the cross with the other hexaploid wheat *T. spelta* did not produce similar result either in terms of seed setting or in terms of hybrid seed fertility. The cytological observation made in a few of these hybrids does not explain the reason for this high seed setting and sprouting.

It seems that either the genome of *Ae. triuncialis* used in our experiments was modified to give satisfactory seed setting or the different cultivars of *T. aestivum* used here changed in such a way, in the course of evolution, as to make good seed setting possible. However, nothing can be said with absolute certainty, unless more data are available from experiments using all the subspecies of the *Ae. triuncialis* species complex. Further studies are being made in this direction keeping this in view.

Acknowledgement

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NEMATOLOGICAL STUDY OF CASING SOILS USED FOR MUSHROOM BEDS AND POSSIBILITIES OF CONTROL

By

K. Farkas, I. Koronczy

UNIVERSITY OF HORTICULTURE, DEPARTMENT OF PLANT PROTECTION, BUDAPEST,
"DUNA" HORTICULTURAL CO-OPERATIVE, CSEPEL

The authors obtained six kinds of casing soils from 14 mushroom plants in Hungary. According to the numerical results of their investigations, crushed limestone and pit-sand are insignificant sources of infection. In crushed lime-stone, hotbed soil, peat and medium heavy field soil the authors found 1, 12, 13 and 24 nematode species, respectively. The proportion of mycopathogenic species was 0.0 per cent in crushed lime-stone, 1.14 per cent in peat, 2.98 per cent in hotbed soil and 8.28 per cent in plough-land. The sterilization of casing soils with formalin (4 lit/m^3) did not prove satisfactory under either laboratory or operational conditions (39.21 and 45.71 per cent respectively). 100 cm^3/m^3 doses of special nematicides did not show total efficiency against nematodes either under operative or under laboratory conditions. Their influence on the value of pH was found unimportant. However, in spite of their imperfect nematicide effect, they had a favourable influence on the yield. In large-scale experiments on sterilizing the casing soils at a rate of 200 cm^3/m^3 all preparations used — with the exception of Vapam — showed a total nematocide effect. As regards yield, Trapex, Nemagon, Fumason and Shell DD were found to be the best.

Introduction

The general demands raised on the casing soils used for the champignon beds are found in the works of the following authors: Atkins (1961), Vedder (1961), Kindt (1966), Koronczy—Uzonyi (1969), etc., but there are no data on the nematodes of the casing soils in the mentioned works. On nematological aspects Paesler (1957), Conroy—Blake (1959a, b), Sumenkova (1964), Bukowski (1967), Choleva (1969, 1970), etc. give some information. Mushroom growers and nematologists agree that casing soils are the potential sources of nematode infection. It is quite obvious that the various casing soils should be judged from different standpoints. So far casing soils have not been analysed from nematological aspects in Hungary. The authors' aim was to identify the nematode species occurring in the different casing soils and find the possibilities of control.

Material and method

1. Nematological studies. Six kinds of casing soil were obtained from 14 mushroom plants of Hungary. In the numerical examination the number of samples were 26 of crushed lime-stone, 25 of crushed lime-stone + peat (75 : 25), 1 of pit-sand, 11 of peat, 5 of hotbed soil, and 15 of medium heavy plough-land. 20 g casing soil per sample was weighed out, and the nematodes run for 24 hours with the improved method of BAERMANN (1917). The number of nematodes was determined with a square-net micrometer.

The percentage of nematode-free samples, the average and maximum abundance values of the nematodes per 100 g material were determined.

In the course of our investigations nematode species were determined in 19 samples of crushed lime-stone, 7 samples of peat, 5 samples of hotbed soil and 12 samples of plough-land. The identification of the nematode species collected from pit-sand and from a mixture of peat and sandstone powder was not feasible.

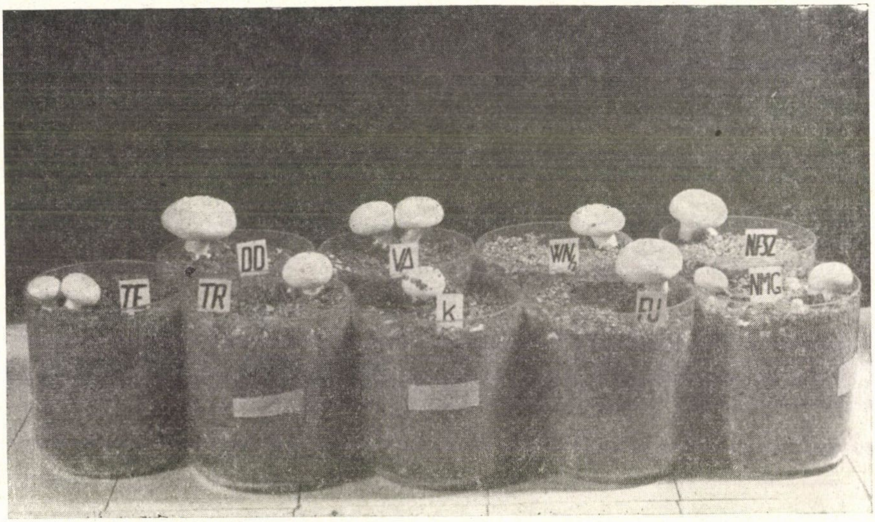

Fig. 1. Experiment on sterilizing casing soils in culture pots (detail)

Besides the identification of the species, the distribution according to sex and the trend of individual dominance (D%) were also studied. The list of nematode species in the different casing soils was analysed on the basis of an ecological classification by PARAMONOV (1952, 1962) too.

2. Control experiments. *a)* Sterilization of casing soils with formalin. As the first step of the control experiments the nematicide effect of casing soil sterilization with formalin, widely used in the Hungarian mushroom plants, was investigated. Laboratory and operative experiments were set up. In both cases a mixture of peat and crushed lime-stone (25 : 75) was sterilized.

Under laboratory conditions the casing soil — one liter per each of ten replications — was sterilized in glass-culture pots. The operative formalin sterilization was carried out with 2 m^3 of casing soil without replication. The dose calculated for 1 m^3 of casing soil was in both cases 4 liter formalin of 40 per cent concentration. The sterilized casing soil was not covered in either of the cases. The number of nematodes was determined before the treatment and 10 days after in 10 replications each under laboratory conditions and in 5 replications under operative conditions.

b) Casing soil sterilization with nematocides. Of the special nematocides the following were used in the experiment; Fumazon (1,2-dibromo-chloro-propane), Nemafos (0,0-diethyl-0-2-pyrazinyl-thiophosphate), Nemagon (1,2-dibromo-chloro-propane), Shell DD cylinders in 5 replications per treatment (Fig. 1). The control tubes were covered with a mixture of non-sterilized peat and crushed lime-stone (25 : 75). The amount of the compost was 70 grams per culture tube.

Under operative conditions the laboratory experiments were repeated with 1/4 m^3 casing soil per treatment. This time, the sterilization was carried out in wooden boxes (Fig. 2), and the sterilized casing soil was covered with plastic film. The number of nematodes was determined before the treatment and 12 days after in 5 replications per treatment.

The preparations applied did not prove to be of total efficiency either under laboratory or operative conditions, therefore the experiments were repeated with an increased dose (200 cm^3) under operative conditions.

A mixture of peat and crushed lime-stone (25 : 75) was sterilized in this series of experiments too. Sterilization was carried out — as in the previous experiments — with 1/4 m-casing soil per treatment placed in wooden cases, without replications. The number of nematodes was established before the sterilization and 12 days after, on the basis of data obtained in 5 replications per treatment.

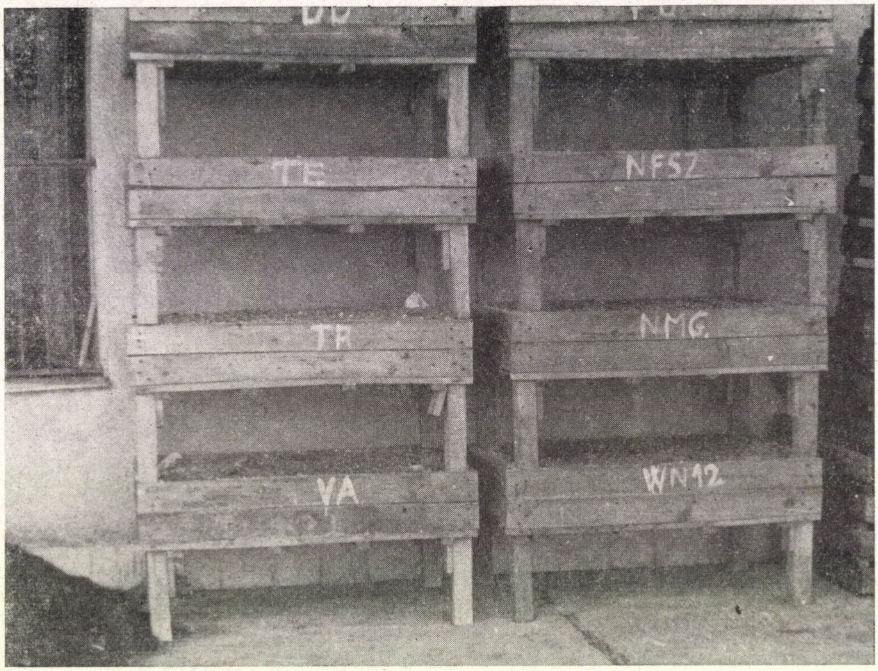

Fig. 2. Large-scale experiment on sterilizing casing soils (detail)

The sterilized casing soils were stored over 58 days at 20—25°C, and in the meantime turned over twice to decrease the fungitoxic effect. On the 58th day wooden cases of 1 m^2 surface area filled with pasteurized compost interwoven with the mycelium D 13 were covered with the sterilized casing soils. The control cases were covered with a mixture of non-sterilized peat and crushed lime-stone (25 : 75). The cases were arranged at random in an above-ground culture house. The amount of mushrooms picked was recorded according to treatments on each occasion.

Results

1. Nematological studies. On the basis of the results obtained in quantitative nematological examinations of casing soils (Table 1) the conclusion was drawn that crushed lime-stone is an unimportant source of infection. The pro-

Table 1

Trends in the nematode infection of various casing soils

Casing soil	Crushed lime-stone + peat	Crushed lime-stone	Pit-sand	Peat	Hotbed soil	Medium heavy plough-land
Number of samples examined	25	26	10	11	5	15
Percentage of nematode-free samples	28	96	90	18	0	6
Average number of nematodes (per 100 g)	334	9	8	409	430	431
Maximum number of nematodes (per 100 g)	3840	240	80	1520	500	1440

portion of nematode-free samples was 96 per cent, the average abundance was 9 nematodes per 100 g material.

The corresponding values of pit-sand too seem to be favourable. In spite of this fact it is not considered a preferred casing soil. Hotbed soil and plough-land appear to be the most dangerous sources of infection. In the case of hotbed soils no nematode-free sample occurred, and the abundance value per 100 g casing soil was also high (430 nematodes). The average abundance value of nematodes in plough-land (431) is the highest of all. The proportion of nematode-free samples (6 per cent) is also unfavourable. The corresponding data of the mixture of peat and crushed lime-stone as well as of peat occupy an intermediate place.

Species isolated from crushed lime-stone (Table 2) agree with the previous data and with the investigation made by Cayrol (1962) in proving that the crushed stone is an unimportant source of infection. Thus it is not by chance that the Hungarian mushroom growers have been giving preference to this casing soil for decades.

13 species of 14 genera were isolated from peat (Table 3). As regards individual dominance, *Rhabditis* sp. (42.52 per cent) and *Cephalobus* sp. (26.43 per cent) show the highest dominance values. Of the species the dominance value of *Enchodorella murithi* (Altherr), Siddiqui was found to be the highest (9.19 per cent), followed by the 3.44 per cent individual dominance value of *Acrobeles ciliatus* Linstow. The species proved by Hooper (1962) to destroy mycelia are only represented by *Aphelenchus avenae* Bastian (with a dominance value of 1.14 per cent).

From plough-land 24 species of 25 genera could be isolated (Table 4). The highest dominance values were found in the *Eudorylaimus* sp. (19.10 per cent) and *Rhabditis* sp. (14.01 per cent). Among the species the highest dominance value was attained by *Aporcelaimellus obscurus* (Thorne et Swanegr) Heyns (10.19 per cent), followed by *Chiloplacus symmetricus* (Thorne) Thoren and *Pratylenchus microdorus* Andrássy, both with an individual dominance

Table 2

Individual dominance of nematode species in crushed lime-stone

Species	Number	Individual dominance (D%)
1. *Rhabditis* sp.	16	94.11
2. *Panagrolaimus rigidus*	1	5.88
Total:	17	99.99

Table 3

Individual dominance of nematode species in peat

Species	Number	Individual dominance (D%)
1. *Pelodera teres*	2	2.29
2. *Rhabditis* sp.	37	42.52
3. *Mesorhabditis spiculigera*	2	2.29
4. *Mesorhabditis monohystera*	1	1.14
5. *Cephalobus nanus*	1	1.14
6. *Cephalobus* sp.	23	26.43
7. *Acrobeles ciliatus*	3	3.44
8. *Chiloplacus demani*	1	1.14
9. *Helicotylenchus multicinctus*	1	1.14
10. *Filenchus filiformis*	1	1.14
11. *Criconemoides kirjanovae*	1	1.14
12. *Aphelenchus avenae*	1	1.14
13. *Eudorylaimus obesus*	1	1.14
14. *Eudorylaimus* sp.	2	2.29
15. *Xiphinema* sp.	1	1.14
16. *Enchodorella murithi*	8	9.19
17. *Alaimus primitivus*	1	1.14

value of 5.09 per cent. Species generally known as mycoparasitic are represented by *Aphelenchus avenae* Bastian (D = 6.36%), ***Aphelenchoides helophilus*** (de Man) Goodey (D = 1.27%) and *Paraphelenchus pseudoparietinus* (Micoletzky) Micoletzky (D = 0.63%).

From hotbed soil 12 species of 17 genera were demonstrated (Table 5). The dominance value of *Rhabditis* species was 44.77 per cent, that of the *Eudorylaimus* sp. 10.44 per cent. Among the species *Tylenchorhynchus* dubius

Table 4

Individual dominance of nematode species in medium heavy plough-land

Species	Number	Individual dominance (D%)
1. *Rhabditis axei*	1	0.63
2. *Rhabditis* sp.	22	14.01
3. *Panagrolaimus rigidus*	4	2.54
4. *Cephalobus persegnis*	7	4.45
5. *Cephalobus* sp.	1	0.63
6. *Eucephalobus oxyuroides*	2	1.27
7. *Acrobeloides* sp.	2	1.27
8. *Chiloplacus symmetricus*	8	5.09
9. *Plectus rhizophilus*	1	0.63
10. *Filenchus filiformis*	2	1.27
11. *Pratylenchus microdorus*	8	5.09
12. *Pratylenchus pratensis*	3	1.91
13. *Pratylenchus* sp.	1	0.63
14. *Tylenchorhynchus* sp.	1	0.63
15. *Helicotylenchus multicinctus*	2	1.27
16. *Aphelenchoides helophilus*	2	1.27
17. *Aphelenchus avenae*	10	6.36
18. *Paraphelenchus pseudoparietinus*	1	0.63
19. *Criconemoides kirjanovae*	1	0.63
20. *Eudorylaimus pratensis*	6	3.82
21. *Eudorylaimus obesus*	5	3.18
22. *Eudorylaimus monohystera*	1	0.63
23. *Eudorylaimus* sp.	30	19.10
24. *Mesodorylaimus* sp.	4	2.54
25. *Enchodorella murithi*	1	0.63
26. *Aporcelaimellus obscurus*	16	10.19
27. *Aporcelaimellus obtusicaudatus*	3	1.91
28. *Aporcelaimellus* sp.	3	1.91
29. *Discolaimus brevis*	1	0.63
30. *Mylonchulus brachyuris*	3	1.91
31. *Mononchus papillatus*	2	1.27
32. *Nygolaimus* sp.	1	0.63
33. *Alaimus primitivus*	2	1.27
Total:	157	99.83

Table 5

Individual dominance of nematode species in hotbed soils

Species	Number	Individual dominance (D%)
1. *Rhabditis terricola*	1	1.49
2. *Rhabditis* sp.	30	44.77
3. *Mesorhabditis* sp.	1	1.49
4. *Caenorhabditis dolichura*	1	1.49
5. *Diplenteron colobocercus*	1	1.49
6. *Panagrolaimus rigidus*	1	1.49
7. *Cephalobus* sp.	5	7.46
8. *Eucephalobus oxyuroides*	1	1.49
9. *Acrobeles* ciliatus	2	2.98
10. *Chiloplacus symmetricus*	2	2.98
11. *Teratocephalus* sp.	4	5.97
12. *Ditylenchus myceliophagus*	1	1.49
13. *Tylenchorynchus dubius*	6	8.95
14. *Aphelenchus avenae*	1	1.49
15. *Nygolaimus* sp.	1	1.49
16. *Eudorylaimus* sp.	7	10.44
17. *Aporcelaimellus obtusicaudatus*	1	1.49
18. *Alaimus primitivus*	1	1.49
Total:	67	99.94

(Bütschli) Filipjev showed the highest dominance value (8.95%). The mycoparasitic species are represented by *Ditylenchus myceliophagus* Goodey (D = 1.49%) and *Aphelenchus avenae* Bastian (D = 1.49%). On the basis of an ecological classification made by PARAMONOV (1952, 1962), the ecological characteristics of nematodes in crushed lime-stone (Table 3) are the following: 94.11 per cent eusaprobionta, 5.89 per cent devisaprobionta. In peat five ecological groups occurred (Fig. 3). The proportion of eusaprobionta was 49.45 per cent, that of devisaprobionta 32.18 per cent; pararhizobionta occurred in 13.79, phytopathogenic species in 3.44 and mycoparasites in 1.14 per cent. Five ecological groups were found in plough-land too (Fig. 3). The proportion of pararhizobionta was 48.40 per cent, that of eusaprobionta 15.94 per cent; devisaprobionta existed in 15.92, phytopathogenic species in 11.46 and mycoparasites in 8.28 per cent. The number of ecological groups found in the hotbed soil was similarly five (Fig. 3). Eusaprobionta showed a proportion of 52.23 per cent, devisaprobionta 22.38 per cent, pararhizobionta occurred in

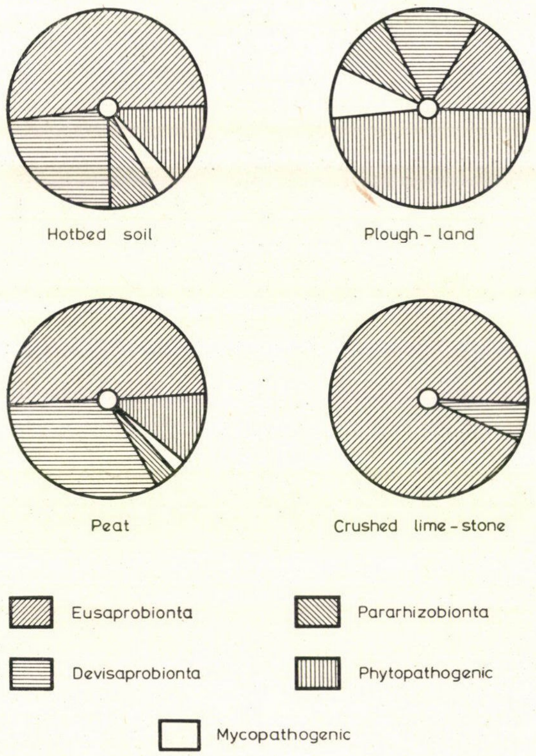

Fig. 3. Ecological characteristics of nematodes in various casing soils

13.43, phytopathogenic species in 8.98 per cent, while the proportion of mycoparasites was 2.98 per cent.

2. Control experiments. The effect of nematocides on the pH-value of the casing soil (Table 6) showed no significant differences in any of the treatments. The lowest deviation (±0.0, ±0.1) was found with Nemafos, the highest (+0.2 and +0.3) with Trapex and Vapam. The results unequivocally prove that the above nematocides applied at a rate of 100 cm^3 increase — though not significantly — the pH value of the casing soil. The efficiency of casing soil sterilization experiments performed with formalin (4 litre/m^3 casing soil applied at a concentration of 40 per cent) under laboratory and operative conditions was found to be 39.21 and 45.71 per cent, respectively, as calculated with Abbot's formula. Thus, the nematocide effect of formalin treatments used in the Hungarian mushroom plants is not satisfactory. This statement is confirmed by the results of Tóth (1968).

The killing effect of 100 cm^3 doses of nematocides as tested under laboratory and operative conditions (Table 7) is highly favourable. Nemafos and Di Trapex were of total effect under laboratory conditions, but under operative

Table 6

Effect of various nematicides (100 cm³/m³) on the pH-value of the casing soil

Nematicide	pH				Deviation	
	Before treatment		After treatment			
	H_2O	KCl	H_2O	KCl	H_2O	KCl
Fumason	7.4	7.2	7.6	7.4	+0.2	+0.2
Nemafos	7.4	7.2	7.4	7.3	±0.0	+0.1
Nemagon	7.4	7.2	7.5	7.4	±0.1	+0.2
Shell DD	7.4	7.2	7.5	7.4	+0.1	+0.2
Telone	7.4	7.2	7.5	7.4	+0.1	+0.2
Trapex	7.4	7.2	7.6	7.5	+0.2	+0.3
Vapam	7.4	7.2	7.6	7.5	+0.2	+0.3
Di Trapex	7.4	7.2	7.5	7.4	+0.1	+0.2

Table 7

Trends in the nematocide effect of various nematicides (100 cm³/m³) under laboratory and operative conditions

Treatment	Laboratory	Operative
	efficiency	
Fumazon	67.80	76.54
Nemafos	100	84.35
Nemagon	73.89	83.65
Shell DD	99.60	94.43
Telone	80.32	80.74
Trapex	99.82	94.55
Vapam	82.86	81.82
Di Trapex	100	97.84
Control	—	—

conditions none of the preparations proved to be of 100 per cent efficiency. The favourable influence of Nemafos confirms the results obtained by Oliff (1965), Hesling (1966), Burton (1969), Hesling—Kempton (1969). The effect on the yield of casing soil sterilization with a dose of 100 cm³ under laboratory conditions showed favourable trends with the exception of Nemagon (Fig. 4). Yield per 1 m² surface and 1 q manure exceeded the average of the control. The best result was obtained by treatments with Trapex; the yield was 8.96 kg/m², i.e. 3.36 kg more than that of the control. The first mushrooms

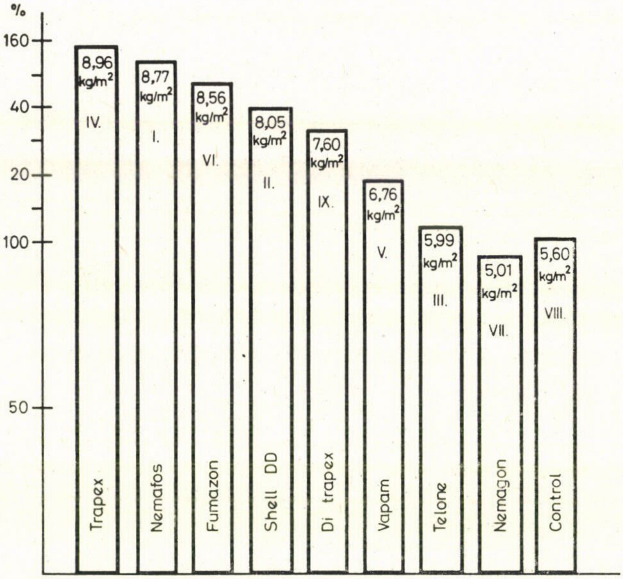

Fig. 4. Effects on yield and on the sequence of yield (I—IX.) of casing soil sterilization carried out with various nematocides (100 cm^3/m^3) under laboratory conditions

appeared in culture pots treated with Nemafos. Mushrooms were picked the latest from culture pots treated with Di Trapex. Fungitoxic effects were not observed in any of the treatments, on the contrary, the mycelium very soon overgrew the casing soil treated with nematocides.

The first mushrooms picked were examined for taste too. An unpleasant taste was only experienced with mushrooms grown in culture pots treated with

Table 8

Trends in the nematocide effect of operative casing soil sterilization (200 cm^3/m^3)

Treatment	Efficiency %
Fumazon	100
Nemafos	100
Nemagon	100
Shell DD	100
Telone	100
Trapex	100
Vapam	99.53
Di Trapex	100
Control	—

Di Trapex. The efficiency of casing soil sterilization with 200 cm^3/m^3 doses of nematocides under operative conditions was hundred per cent (Table 8) with the exception of Vapam. The results of Table 9 show that Trapex, Nemagon, Fumason and Shell DD increase the yield. E.g., in pots treated with Trapex 4.10 kg more mushrooms were produced than in the control plots. It is very important, and disagrees with the laboratory results and those obtained by OLIFF (1965), HESLING—KEMPTON (1969), that the first mushrooms produced in pots treated with Nemafos were found to be distorted, and even the total crop result was outstandingly low, being more than 50 per cent less than that of the control. An unpleasant taste was only observed in the case of mushrooms produced in pots treated with Di Trapex.

Table 9

Effect on yield of casing soil sterilization carried out with various nematocides (200 cm^3/m^3) under operative conditions

Treatment	Total yield, kg	Yield, kg/m^2
Fumason	50.15	10.3
Nemafos	21.00	4.20
Nemagon	50.25	10.05
Shell DD	49.43	9.88
Telone	45.35	9.07
Trapex	51.30	10.26
Vapam	44.30	8.86
Di Trapex	39.45	7.89
Control	47.20	9.44
S.D.$_{5\%}$ = 1.58		

Conclusions

On the basis of quantitative nematological examinations of casing soils the conclusion can be drawn that crushed lime-stone and pit-sand are unimportant sources of infection. Plough-land, hotbed soil and peat can be regarded as dangerous, even if only the numerical data are considered. These statements are confirmed by the results of studies on the species as well. Not a single mycoparasitic species occurs in crushed lime-stone. In peat one, in hotbed soil two and in plough-land three mycoparasitic species were found. Plough-land seems to be the most dangerous source of infection, as is also suggested by the proportions of ecologic characters, namely, the proportion of mycopathogenic species is 1.14 per cent in peat, 2.98 per cent in hotbed soil and 8.28 per cent in plough-land. In spite of this fact it would be risky to insist on saying that it is really so, as, apart from *Aphelenchoides composticola* Franklin GOODEY (1960) considers *Ditylenchus myceliphagus* Goodey to be the most dangerous mycoparasitic species. And as it was seen, this species was isolated from a glasshouse soil! After all, it can be said that the Hungarian mushroom growers were unconsciously right in choosing crushed stone and a mixture of crushed stone and peat (75 : 25) for the purpose of casing soil. As a final conclusion of the investigations made on the other two casing soils, it can be said that when hotbed soil or plough-land soil are to be used as casing soils the control of nematodes must not be neglected.

In laboratory and operative experiments of casing soil sterilization the nematocide effect of the 40, per, cent formalin treatments widely applied by the Hungarian growers was pointed out to be unsatisfactory.

The eight special nematocide preparations tested under operative and laboratory conditions do not provide a full protection against nematodes when applied in a dose of 100 cm^3. They increase — though not considerably — the pH value of the casing soil (± 0.0, ± 0.3). According to culture pot experiments performed under laboratory conditions, the yield may show favourable trends in spite of a nematocide effect below 10 per cent. According to the evidence of the operative experiments, the 200 cm^3-dose results in a total nematocide effect, but this is not accompanied by an increased yield in the case of all preparations. Trapex proved to be the most efficient, followed by Nemagon, Fumason and Shell DD. The order of succession is similar to that found in the laboratory experiments. The most essential difference is that Nemafos applied in a dose of 200 cm^3 causes deformations in the first mushrooms produced and reduces the yield considerably. It seems to be a general phenomenon that the mycelium grows through the sterilized casing soil in a short time. Di Trapex causes unfavourable changes in the taste.

As a final conclusion it can be said that: 1. casing soils properly chosen (crushed stone, or a mixture of crushed stone and peat) provide in themselves a sufficient protection; 2. with Trapex, Nemagon, Fumason and Shell DD, — that is preparations of dichlorine and dibromine content, the casing soil can be efficiently sterilized and the yield increased. Further investigations, first of all on chemical residues, cannot — naturally — be dispensed with.

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EFFECT OF BENZIMIDAZOLE AND ITS DERIVATIVES ON THE INTENSITY OF PHOTOSYNTHETIC CARBON DIOXIDE FIXATION IN ALFALFA AND MAIZE LEAVES

By

L. HORVÁTH, B. I. POZSÁR

ISOTOPE INSTITUTION OF THE HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST;
INSTITUTE OF AGROBOTANY, TÁPIÓSZELE

The authors demonstrated a considerable increase in the intensity of photosynthetic carbon-dioxide fixation under the influence of benzimidazole and its derivatives at an early stage of the treatment when the stimulation of the intensity of protein synthesis could already be pointed out, but no biological effectivity increasing the protein level was yet observed. It is worth being emphasized that stimulation caused by the primary base is higher than the effect of derivates. The stimulation caused by benzimidazole is much higher in maize leaves than in those of alfalfa, as related to its specific activity. The authors have arrived at the conclusion that the stimulation of photosynthetic carbon-dioxide fixation by the benzimidazole-type resistance factors (systemic fungicides) is one of the favourable by-effects of bioactive compounds beside the stimulation of protein synthesis.

Introduction

In earlier papers we gave a detailed account of the stimulatory effect of synthetic cytokinins on photosynthetic carbon-dioxide fixation (POZSÁR 1967, 1971) and raised the idea that cytokinins, beside inhibiting senescence, exert an effect on the preservation of chlorophyll. These latter processes were interpreted partly as senescence inhibition, partly as a rejuvenating effect (KIRÁLY–POZSÁR–EL HAMMADY 1966). We also disputed alent references to the intensity of photosynthetic carbon-dioxide fixation (POZSÁR 1971), emphasizing that in the extreme cases of chlorophyll decomposition, as well as under the specially stimulated biological mechanism of protein synthesis, the intensity of photosynthetic carbon-dioxide fixation also shows a characteristic though atypical modification. In pathological and onthogenetical processes the biological activity of leaf tissues cannot be sufficiently characterized by the chlorophyll content, while at the same time, with the intensity of photosynthetic carbon-dioxide fixation related to the protein content — more closely to the soluble (in 0.5 per cent NaCl) protein content, — a direct and positive correlation can be pointed out (POZSÁR 1971). It is highly probable that the enzyme system of the Calvin cycle is dissolved with the salt solution resulting in the correlation.

In another paper we described that the benzimidazole treatment stimulated the intensity of the protein synthesis and increased the protein content in the leaves (Pozsár—Király—El Hammady 1967). On this basis it is considered highly probable that benzimidazole as well as many of its bioactive derivatives (Pozsár 1972) also stimulate the intensity of photosynthetic carbon-dioxide fixation.

Material and method

In this paper we present the results of photosynthetic carbon-dioxide fixation in alfalfa and maize leaves, as determined after a previous treatment in bioactive solutions. Discs excised from the leaf tissues of test plants were floated for 18 hours in a solution of 200 ppm concentration. The intensity of photosynthetic carbon-dioxide fixation was calculated from the fixation data obtained in light and dark, after an exposure of 15 minutes. Of barium carbonate of 130 mCi/g specific activity 120 microCi activity was used for each of the experiments in a special apparatus by means of which air temperature, atmospheric pressure, inactive and active carbon-dioxide content in the experimental space, as well as wavelength and intensity of light could be standardized. Temperature was fixed at 22 °C by means of an ultrathermostat. The radioactivity of tissues was determined in a Packard Tri Carb apparatus by the method of liquid scintillation (Horváth—Bursics—Márton—Pozsár 1972).

The other details of the method were described in previous papers. The intensity of photosynthetic carbon-dioxide fixation was expressed as a specific activity related to chlorophyll, and, for the sake of an easier comparison, stimulation was given in the tables in percentages too.

Results

The benzimidazole derivatives decidedly stimulated the intensity of photosynthetic carbon-dioxide fixation in the leaf tissues of the studied mono- and dicotyledonous test plants. Table 1 shows the stimulation of lucerne leaves under the influence of benzimidazole, 2-amino-benzimidazole, 5,6-dimethyl-benzimidazole, "Benlate" and "Thiabendazole", in the case of short exposures. In the case of a short exporuse the stimulatory effect of the tested benzimidazole derivatives on the intensity of protein synthesis and increase of protein level cannot be demonstrated as yet, at the same time, the treatment considerably stimulates the intensity of photosynthetic carbon-dioxide fixation as related to the specific activity. It deserves mentioning that the basic substance induces a more favourable stimulation than its derivatives, whose distinguished biological activity in other directions is generally known. The biological effectivity of 5,6-dimehtyl-benzimidazole — according to the bioassay performed — cannot be regarded as proved.

The influence of treatments with benzimidazole and 2-amino-benzimidazole on the intensity of photosynthetic carbon-dioxide fixation in the leaf tissues of maize is shown in Table 2. According to the data of the table the stimulatory effect of the basic substance is much higher than that of the derivative, which agrees with the results obtained in the case of papilionaceae. Stimulation caused by the benzimidazole relative to the specific activity is

Table 1

Effect of benzimidazole derivatives on the intensity of photosynthetic carbon-dioxide fixation in alfalfa leaves, as related to 100 mg fresh weight

Benzimidazole derivative	Carbon-dioxide fixation cpm/100 mg			Chlorophyll content mg/100 mg fresh weight	Specific activity	
	in light	in dark	photo-synthetic		cpm/mg chlorophyll	%
Control	185.2	12.8	172.4	0.72	238.9	100
Benzimidazole	246.7	16.4	230.3	0.81	284.3	118
2-amino-benzimidazole	221.5	18.5	203.0	0.76	267.1	113
5,6-dimethyl-benzimidazole	192.3	21.5	170.8	0.70	244.0	102
"Benlate"	234.6	17.0	217.6	0.85	256.0	107
"Thiabendazol"	228.9	19.3	209.6	0.77	272.2	114

Table 2

Effect of benzimidazole and its derivative on the intensity of photosynthetic carbon-dioxide fixation in maize leaves, as related to 100 mg fresh weight

Bioactive compound	Carbon-dioxide fixation cpm/100 mg			Chlorophyll content mg/100 mg fresh weight	Specific activity	
	in light	in dark	photo-synthetic		cpm/mg chlorophyll	%
Control	335.8	21.7	314.1	0.89	352.6	100
Benzimidazole	618.6	48.5	550.1	1.21	454.6	128
2-amino-benzimidazole	452.1	33.6	418.5	1.03	405.8	115

much higher in maize than in alfalfa. This character is in close agreement with our earlier experiences in as much as the biological effectivity of synthetic cytokinins is much higher in the papilionaceae than in the grasses, while, at the same time, the action of benzimidazole is exceedingly high on the grasses compared to the papilionaceae (POZSÁR—KIRÁLY—EL HAMMADY 1967).

A by-effect — beside the stimulation of the intensity of protein synthesis and effectivity in increasing the protein level — of benzimidazole-type resistance factors (systemic fungicides) has become easy to characterize by the photosynthetic carbon-dioxide fixation.

The considerable difference in biological effectivity between the two test plants examined can obviously be explained by their photorespiration differences, namely, the photorespiration of alfalfa is negligible, while that of the maize is intensive. The latter aspect is not confirmed as yet by direct experimental data.

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EFFECT OF DEGRANOL ON STOMA FORMATION IN THE COTYLEDON OF ALLIUM CEPA L.

By

P. Balogh, Á. Keresztes

COUNTY HOSPITAL, SALGÓTARJÁN; DEPARTMENT OF APPLIED BOTANY AND HISTOGENETICS OF THE EÖTVÖS LORÁND UNIVERSITY, BUDAPEST

The authors performed light-microscopic studies on the effect of Degranol solutions of 10^{-4} M—10^{-2} M concentration on the differentiating epidermis of onion seedlings. They found that increasing the concentration of the solutions the number of stomata-per-unit area decreased and the persisting stoma mother cells became abnormally large, their nuclei enlarging as well. The shape of the surrounding epidermis cells did not significantly differ from the control. The authors compared the results with those obtained in colchicin-treated onion epidermis on the one hand, and in animal and human tissue cultures treated with Degranol, on the other.

Introduction

The developing foliar leaf and cotyledon of *Allium cepa* L. are highly suitable test materials for studying two important steps of cell differentiation, namely the formation of stoma mother cells and stomata (Strasburger 1866, Bünning—Biegert 1953, Fridvalszky 1957). Fridvalszky—Keresztes (1966) described three differentiation zones on the cotyledon and characterized them cytologically. At the bottom of the first (basal) zone the cells divide equally, at the growth rate of the cotyledon; the development of the stoma mother cells takes place on the border of zones 1 and 2 by inequal division. On the border of zones 2 and 3 the stoma mother cells divide equally into two guard cells, so the stomata develop. Keresztes—Fridvalszky (1967) inhibited the stoma formation by germinating in colchicin solution, and at the same time pointed out the disturbance of cell polarity both on a microscopic and a submicroscopic level. In this context it seemed interesting to study the stoma formation under the influence of a cytostatic substance (Degranol) that had a chemical structure other than that of colchicin.

Material and method

Seeds of *Allium cepa* L. (onion, Makói Elit, I. class) were germinated in Degranol solutions of different (10^{-4} M—10^{-2} M) concentration. (The Degranol = 1,6-bis-(2-chloro-aethylamino)-1,6-dideoxy-D-mannitol-dihydrochloride, is a cytostatic drug successfully used in the chemotherapy of certain malignant diseases; as to its action, it belongs to the group of the alkylating agents.) Germination was carried out at room temperature, in petridishes, on filter paper. The epidermis of the cotyledon of 5—10- days- old seedlings was prepared by stripping and studied under light-microscope. (The germination period was changed within 5—10 days according to the extent of growth inhibition.) The micrographs were taken by a Zeiss automatic exposing equipment on ORWO NP 15 film. The cells were counted by means of a thrombocyte ocular.

Results

In the control material stomata consisting of two bean-shaped guard-cells without subsidiary cells develop well below the bend of the cotyledon (Fig. 1). The development of the stomata was also studied on the treated plants around the bend of the cotyledon. As a response to the treatment, the frequency of stoma formation was found to decrease with the increase of concentration. The Table presents the averages of measurements taken after three treatments with characteristic effects. The solution of 10^{-4} M concentration significantly reduces the number of stomata per unit area, among which the persistent (not dividing) stoma mother cells appear. In the case of a 10^{-3} M concentration the latter become dominant, while in a $2 \cdot 10^{-3}$ M solution the number of stoma mother cells also decreases considerably. In solutions of higher concentrations stomata can only be found very rarely, and the number of stoma mother cells continues to decrease.

Table 1

Changes in the frequency of stoma mother cells and stomata around the bend of cotyledon under the influence of Degranol

	Number of stoma mother cells $\bar{x} \pm S_{\bar{x}}$	Number of stomata $\bar{x} \pm S_{\bar{x}}$
Control	0	137±2.9
$1 \cdot 10^{-4}$ M	5±2.0	102±4.8
$1 \cdot 10^{-3}$ M	105±8.4	40±6.8
$2 \cdot 10^{-3}$ M	44±5.1	5±2.0

The data refer to an area of 1 mm², and each group means the average of 20 measurements. P (the probability of random value) is lower than 4 per cent in each group

In the course of our investigations various abnormities of stoma mother cells and stomata were encountered. The nuclei of stoma mother cells frequently enlarge compared to the nuclei of the surrounding epidermal cells, and the cell dimensions also grow significantly (Fig. 2). Sometimes even an extreme increase of size could be observed (Fig. 3). The fact that they are really stoma

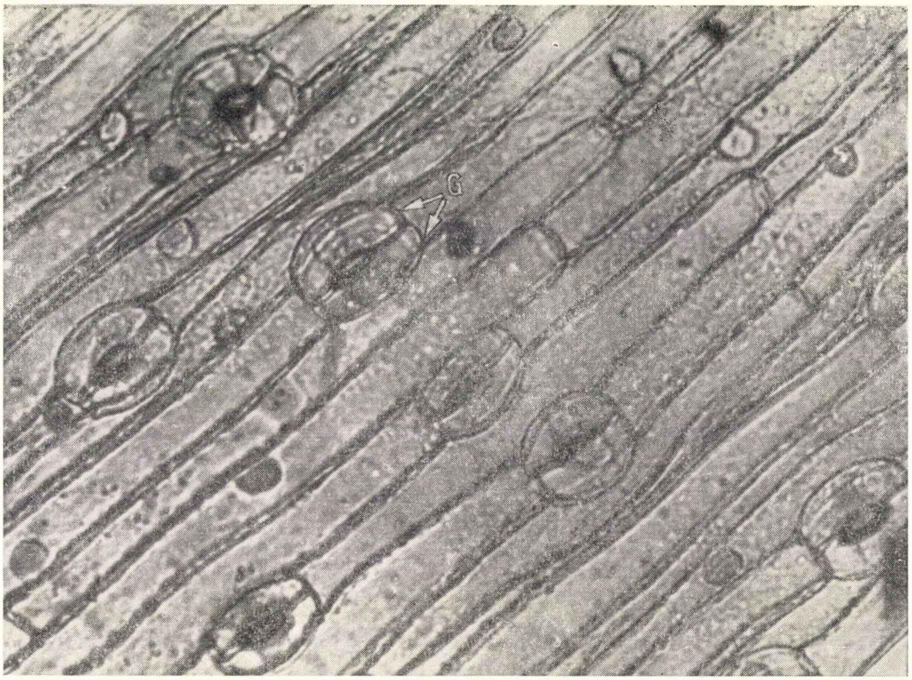

Fig. 1. Epidermis of the cotyledon in an untreated seedling (G = guard cell) ×400

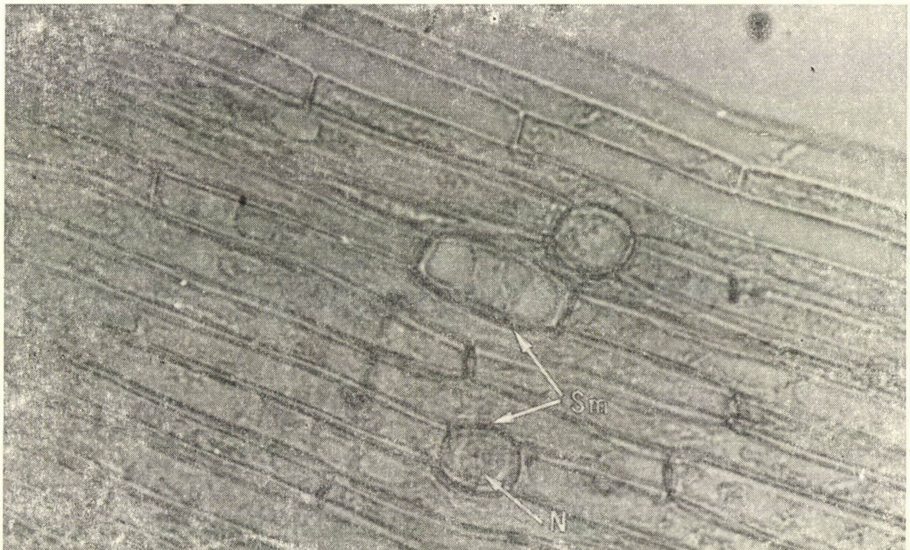

Fig. 2. Epidermis of the cotyledon in a seedling treated with $2 \cdot 10^{-3}$ M Degranol solution (Sm = stoma mother cell, N = nucleus) ×400

Fig. 3. Epidermis of the cotyledon in a seedling treated with $2 \cdot 10^{-3}$ M Degranol solution (Sm = stoma mother cell, N = nucleus, W = partially developed cell-wall) ×400

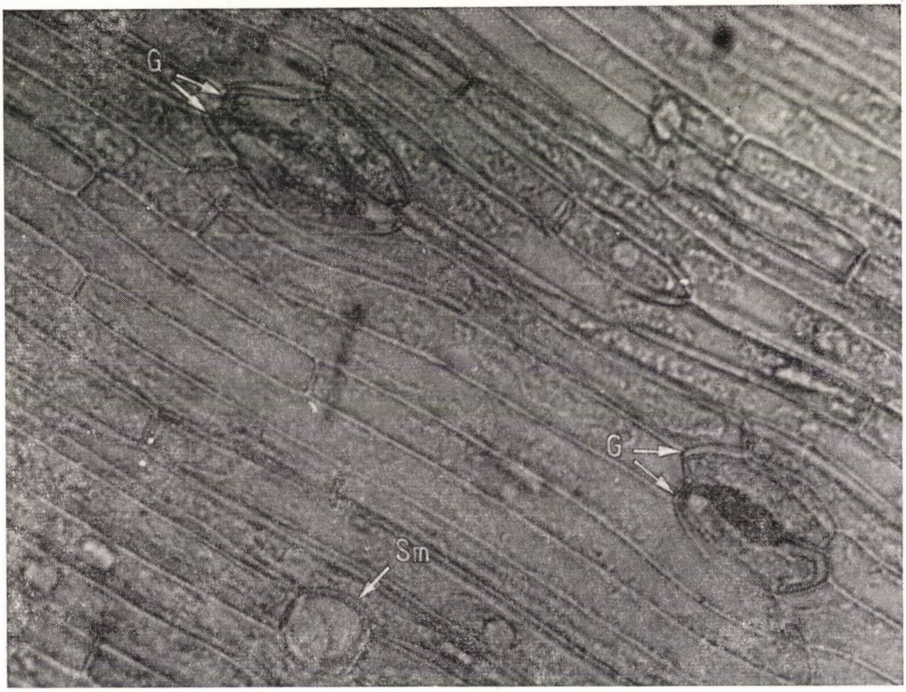

Fig. 4. Epidermis of the cotyledon in a seedling treated with $2 \cdot 10^{-3}$ M Degranol solution (Sm = stoma mother cell, G = guard cell) × 400

mother cells is proved by the imperfect cell division and partial cell-wall formation seen in the cell indicated in Fig. 3. We found giant stoma mother cells coming close in size and shape to the epidermis cells.

The most frequent abnormity of stomata was similarly the increased size (Fig. 4). There occurred asymmetric guard-cell formation, slanting cell-wall insertion, which, however, could also be observed — though very seldom — in the control.

Discussion

As it was mentioned in the "Results", preparation on the treated plants was also made from the bend of the cotyledon; namely, this characteristic part could be recognized even on plants germinated in the solution of highest concentration. The bright green colour of the cotyledons similarly agreed with that of the control. External morphological examinations only revealed differences in the length of the seedlings; the rate of growth decreased with the increasing concentration.

These findings significantly differed from the morphological effect of the $4 \cdot 10^{-3}$ M colchicin solution, exerted on the same object (KERESZTES—FRIDVALSZKY 1967), which manifested itself in a more intensive growth inhibition, chlorophyll deficiency, swelling of meristemic tissues and disappearance of the bend of the cotyledon.

On the cotyledons of colchicin-treated plants all epidermis cells enlarge and become more or less round (probably as the result of polarity disturbance); in plants treated with Degranol the same morphological picture can be observed in a proportion of the persistent stoma mother cells. Since under the influence of Degranol the epidermis cells do not lose their narrow elongated shape, no disturbance of cell polarity can be supposed to exist here; the giantism of stoma mother cells can probably be attributed to the effect of endopolyploidy. The absence of stoma formation in Degranol solutions of higher concentration thus does not mean the inhibition of DNA synthesis but of the subsequent mitosis. This agrees with the observation that in animal and human tissue cultures Degranol blocks the cell cycle in the G_2 phase (PÁLYI—GYESKÓ—SUGÁR 1969, GYESKÓ—SUGÁR 1970, SUGÁR—GYESKÓ 1970).

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CYTOLOGICAL STUDY OF THE EFFECT OF SOME MUTAGENIC AGENTS IN PEAS (PISUM SATIVUM L.)

By

VO HUNG, B. NAGY-PORPÁCZY

UNIVERSITY OF HORTICULTURE, DEPARTMENT OF PLANT GENETICS, BUDAPEST

The cytological effect of some mutagenic agents on peas was studied in mitosis and meiosis. The varieties used in the experiment gave different responses to various mutagenic treatments. As a result of a treatment with 0.05 per cent N-nitrosomethyl urea, elongated, deformed cells were found in the variety Gloria di Quimper. In interphase the occurrence of multinuclear cells was observed. Under the influence of 0.1-per-cent ethyl methane-sulphonate the course of cell division was regular but after the 0.3-per, cent treatment cells with 2—3 nuclei were found in three varieties. X-rays of 5 and 10 kr did not cause any disorder in the cell division, but in the case of 15 kr chromosome and chromatide aberrations were observed. After treatments with gamma 5 kr + ethyl-methane-sulphonate of 0.1 per cent concentration groups of chromosomes stuck together were found in the mitosis metaphasis. In interphases cells with 2—3 nuclei occurred. Gamma irradiation at 5, 10, 15, 20 and 25 kr had a strong inhibitory effect on cell division in the varieties Mansfelder Grüne and Zenith. High reactivity to gamma irradiation was found in the varieties Gloria di Quimper and Bärdi which responded with abnormal cell division to each dosis. In the mitosis metaphasis chromosome and chromatide breaking, in the anaphasis chromosome and chromatide bridges were observed. Abnormal cell division occurred in meiosis too. Aberrations in the mitosis and meiosis could be found also in the M_2 generation, though their frequency compared to the M_1 generation decreased considerably.

Introduction

Utilization of mutations induced by physical and chemical agents is becoming more and more general in pea improvement. Peas are very suitable for studying the effect of mutagenes, since they are strictly self-pollinating and genetically well- known plants.

Mutations have been induced by a wide range of treatments. X-ray treatments were used by GELIN (1954), LAMPRECHT (1957), LEWIS (1962) and WELLENSIEK (1964), who found that the treatments greatly inhibited the division of cells. According to the observations of GOTTSCHALK—MILUTINOVIC (1970) in some 25 per cent of the studied cells the chromosomes formed a ring in meiosis, and normal mitosis did not occur.

PYATENKO (1970) pointed out that after gamma irradiation the frequency of abnormal mitosis depended on the degree of radiosensitivity. The higher the dosis applied, the more frequent the aberration; according to PETKOV

(1969) with an increase in the frequency of aberrations germinability decreases. Kurnik (1969) obtained polyploids after gamma-irradiation (20 kr). Vasileva — Mehandyiev (1971) studied the radiosensitivity of the varieties and found it highly diversified.

Chemical mutagens are widely used from year to year as well. Heringa (1964), Speckmann (1964), Chernov (1965), Bálint *et al.* (1968) used ethyl-methane-sulphonate to induce mutation. Heringa (1964), Wellensiek (1964), Sharma (1966), Chernov (1965), Dolgih (1970) found the mutagenic effect of ethyl-methane-sulphonate to be higher than that of ionizing irradiation. According to Narsinghani — Kumar (1971), treatments with ethyl-methane- and methyl-methane-sulphonate inhibited the development of pea seedlings and caused chromosome aberrations. Zoz — Makarova (1965) found a higher frequency of aberrations in the case of nitrosomethyl urea applied than in nitrosoethyl urea treatments.

As to combined treatments, relatively few literary data are available. Akopyan (1967) studied the percentage of chromosome aberrations as a response to various-type ionizing irradiations and ethyl-methane-sulphonate treatments, and found that in the case of combined treatments the frequency of chromosome aberrations decreased. Similar results were obtained by Mehandyiev (1969).

In the literature in general it is only in relation to a certain variety and a certain mutagenic agent that an author presents his results. Comparative studies concerning treatments applied to more than one variety with a number of agents are rather incomplete.

The aim of our investigations is to study the cytological effects of some physical and chemical mutagenic agents with special regard to gamma irradiation and the sensitivity of the varieties.

Material and method

The experiments were set up at the Department of Plant Genetics and Breeding of the University of Horticulture. In the experiments the varieties Gloria di Quimper, Mansfelder Grüne (round-seeded peas,) Zenith and Bärdi (marrowfat peas) widely spread in commercial production were used.

1. Chemical treatment. Dry seeds were treated for 12 hours with 0.01-, 0.03- and 0.05-per-cent solutions of nitrosomethyl urea (NMK), and 0.1- and 0.3- per-cent solutions of ethyl-methane-sulphonate (EMS). The control seeds were placed in distilled water during the period of treatment.

2. Physical treatment. Dry seeds were treated with gamma irradiation at 5, 10, 15, 20 and 25 kr, and X-rays of 5, 10, 15 and 20 kr. The treatments were performed by the National Institute of Oncology.

3. Combined treatment. The dry seeds were treated first with gamma irradiation (5 kr) then with a 0.1 per cent aqueous solution of ethyl-methane-sulphonate over 12 hours. The cytological examinations were made in mitosis and meiosis. The material was fixed for 24 hours in carnoy, then placed in a 75 per cent alcohol solution. It was stained with carmine acetic acid, and after heated in a 45 per cent acetic acid, a rubbing preparation was made of it.

The seeds were germinated in petridishes immediately after the treatment. After establishing the germination percentage, root tips were collected for the mitosis study 48 hours after the treatment. 100 seeds were germinated per treatment in each variety of which 25—30 root tips were examined.

For studying the meiosis the material was collected before flowering, when the buds appeared, and examined with the above method. Buds were collected from about 25 plants per variety and treatment.

The microscopic photos were made with the M.B 11 Soviet microscope and Praktika FX_3 camera.

Results

Aberrations were found — though in a minimum quantity (1.1%) — even in the controls of the individual varieties. The number of chromosomes was $2n = 14$, $n = 7$ (Fig. 1).

Fig. 1. Control, mitosis metaphasis $2n = 14$ (1800×)

With N-nitrosomethyl urea (NMK) applied at concentrations of 0.01 and 0.03 per cent no substantial cytological changes could be pointed out in mitosis. Treatments at 0.5-per-cent concentration produced very long deformed cells in the variety Gloria di Quimper. In the varieties Mansfelder Grüne and Zenith the treatment inhibited the cell division. In interphasis the occurrence of shattered and multinuclear cells was observed. On the other hand, cell division in the variety Bärdi showed a normal course.

In the case of treatments with 0.1-per-cent ethyl-methane-sulphonate (EMS) the cell division was normal. A concentration of 0.3 per cent produced cells with 2—3 nuclei in three varieties (Gloria di Quimper, Mansfelder Grüne and Bärdi).

In varieties treated with X-ray of 5 and 10 kr abnormal cell division was not observed, with 15 kr, however, chromosome and chromatide aber-

Table 1

Chromosome aberrations induced by gamma irradiation in mitosis
1971—1972

Treatment	M$_1$				M$_2$			
	number of dividing cells observed	fragmentation, %	bridges, %	total chromosome aberrations, %	number of dividing cells observed	fragmentation, %	bridges, %	total chromosome aberrations, %
Variety: Gloria di Quimper								
Control	374	0.8±0.3	0.3±0.2	1.1	172	0.6±0.3	0.6±0.3	1.2
Gamma 5 kr	345	9.8±2.8	5.7±1.1	15.5	163	1.8±0.5	0.6±0.3	2.4
Gamma 10 kr	253	28.8±1.7	12.2±0.6	11.0	162	2.4±1.0	1.8±0.8	4.2
Gamma 15 kr	257	40.8±3.5	16.7±1.6	57.5	205	5.3±1.3	1.9±0.7	7.2
Gamma 20 kr	262	60.6±1.6	20.9±1.9	81.5	182	6.0±2.3	2.7±0.9	8.7
Variety: Bärdi								
Control	112	0.9±0.3	0.0	0.9	114	0.0	0.0	0.0
Gamma 5 kr	186	10.2±2.2	1.0±0.4	11.2	105	1.8±0.4	0.0	1.8
Gamma 10 kr	205	25.5±2.0	4.4±0.8	29.9	148	5.1±0.6	0.8±0.3	5.9
Gamma 15 kr	101	40.0±1.8	9.9±1.0	49.9	198	4.8±0.5	3.0±0.6	7.8
Gamma 20 kr	210	51.5±2.0	28.0±1.8	79.5	—	—	—	—

rations occurred. In the 20 kr treatment cytological examination could not be performed in lack of material.

Treatments with 5 kr gamma irradiation +0.1 per cent ethyl-methane-sulphonate produced groups of chromosomes stuck together in the mitosis metaphasis in the varieties Gloria di Quimper and Zenith, while in the varieties Mansfelder Grüne and Bärdi multinuclear cells were found in the mitosis interphasis.

Gamma irradiation at 5, 10, 15, 20 and 25 kr had a strong inhibitory effect on cell division in the varieties Mansfelder Grüne and Zenith. The varieties Gloria di Quimper and Bärdi responded with an abnormal cell division to each dosis of gamma irradiation. In mitosis, metaphasis chromosome breaking, in anaphasis, chromosome bridges and chromatide breaking were observed (Figs 2, 3).

With increased doses the number of abnormally dividing cells increased too. In the case of gamma irradiation applied at 15, 20 and 25 kr highly fragmented chromosomes were found in the mitosis metaphasis. A part of the injured chromosome remained in the equatorial plane in ana- and telophasis (Fig. 4). It can be supposed that, owing to the loss or injury of the centromeron, these injured chromosomes cannot be drawn to the poles, therefore such

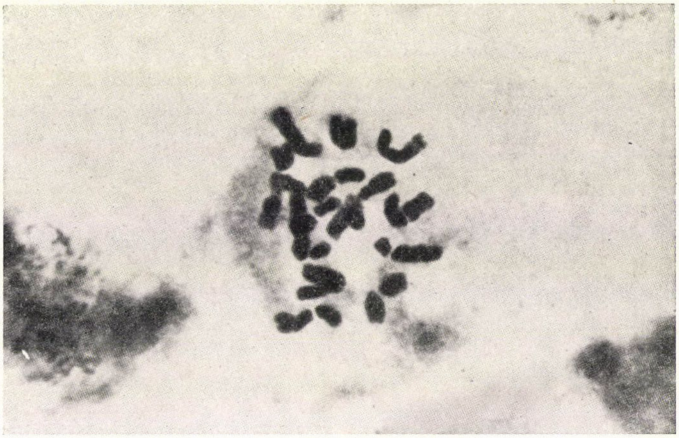

Fig. 2. Mitosis metaphasis, fragmented chromosomes (1800 ×)

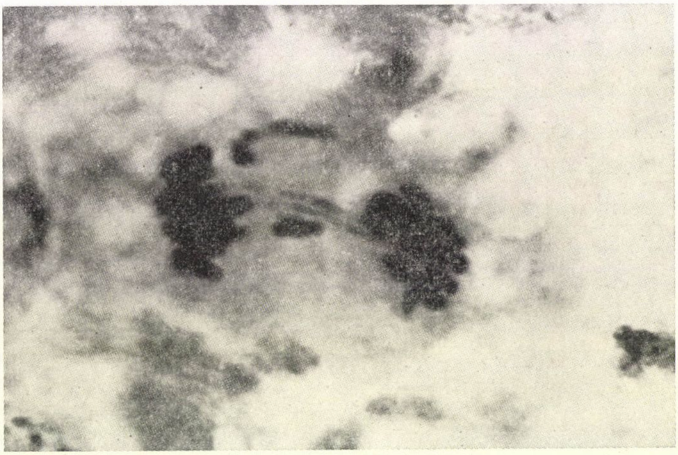

Fig. 3. Mitosis anaphasis with bridge (1800 ×)

Fig. 4. Mitosis anaphasis with chromosomes left behind (1800 ×)

chromosomes and fragments will be eliminated during the further division of cells.

On account of the abnormal division, groups containing more or less chromosomes develop resulting in the appearance of a number of smaller nuclei. In treatments of 10, 15 and 20 kr some plants could successfully be raised in spite of the high rate of abnormal division. In these plants abnormal cell division was shown in the meiosis too. In the meiosis I metaphasis the separation of bivalents was slow, and consequently chromosome aberrations occurred (Fig. 5). In the 25 kr treatment, however, the cells were damaged to such an extent that the seedlings — unable to continue their development — were destroyed (Fig. 6).

Fig. 5. Meiosis I metaphasis with bivalents not easily separating in M_1 (1800×)

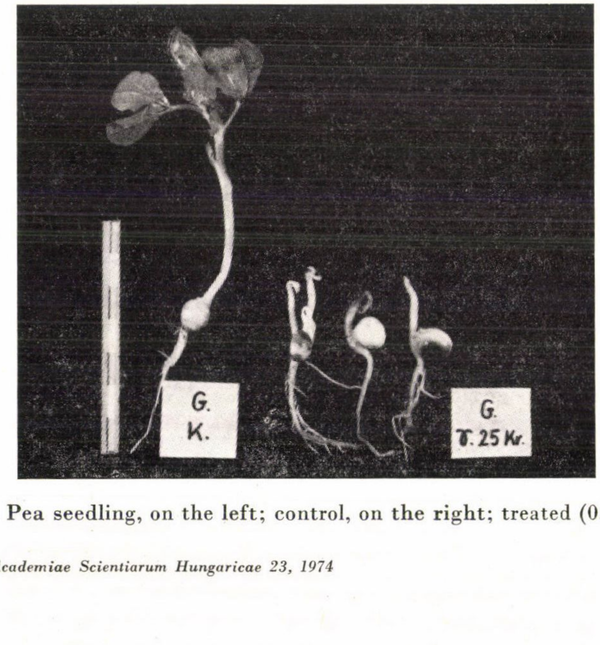

Fig. 6. Pea seedling, on the left; control, on the right; treated (0.25×)

In the M_2 generation the cytological examination was repeated in mitosis and meiosis and the following was found. Gamma irradiation resulted in a repeated abnormal cell division in the varieties Gloria di Quimper and Bärdi. With irradiation of 5 kr the cell division showed a normal course. In the case of irradiation applied at 10, 15 and 20 kr fragmented chromosomes were found in the mitosis metaphasis, and chromosome and chromatide bridges in the anaphasis. In the meta- and anaphasis of meiosis I chromosome and chromatide bridges and rings (translocation) occurred (Figs 7, 8). In meiosis II pentades were found instead of tetrades.

For these varieties in the M_1 and M_2 generations also the percentage proportions of chromosome aberrations occurring under the influence of various treatments were determined in mitosis, as seen in Table 1. The table clearly shows that, the higher the applied dosis, the larger the number of abnormal cells. When comparing the frequency of chromosome aberrations in

Fig. 7. Meiosis I anaphasis chromosomal aberrations in M_2 (1800×)

Fig. 8. Meiosis I anaphasis chromosomal aberrations in M_2 (1800×)

the two generations of the variety Gloria di Quimper it can be established that while in M_1 the 20 kr gamma irradiation resulted in a 81.5-per-cent aberration, in the M_2 plants originating from the same M_1 treatment the percentage of aberration was only 8.7 which suggests that the changed line of cells is selected out during the development, and the further division of cells will be regular. The table shows, further, that the frequency of fragmentation is higher than the occurrence of anaphasis bridges.

When comparing the two varieties we can see that, as regards the frequency of aberrations, there is no essential difference between the two varieties.

Discussion

According to our investigations X-ray treatments of 5—20 kr did not inhibit the cell division, as stated by some authors (Lewis 1962, Wellensiek 1964).

In the case of gamma irradiation the extent of abnormal division depended on the dosis. Similar results were obtained by Pyatenko (1970). After gamma-irradiation Kurnik (1969) found a large number of polyploids in the progeny. Under the influence of the treatments the chromosomes showed structural changes (aberrations), but the chromosome number did not change.

According to Vasileva—Mehandyiev (1971), the varieties display different sensitivity. Similar results were obtained in our own experiments too. Of the varieties used Gloria di Quimper was the most reactive to the treatments.

In our further investigations we should like to find out whether there is any correlation between the chromosome aberrations and the frequency of mutants.

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QUICK DETERMINATION OF CASEIN CONTENT IN PRODUCER'S RAW MILK WITH RESPECT TO PATHOLOGICAL MILKS

By

A. WÁGNER, I. MERÉNYI, M. DOBOS-KOVÁCS

TRUST OF DAIRY ENTERPRISES, DAIRY PRODUCT CONTROLLING STATION, BUDAPEST;
DAIRY ENTERPRISE OF FEJÉR AND KOMÁROM COUNTIES, SZÉKESFEHÉRVÁR

The authors elaborated a quick direct method for determining the casein content of milk. The essence of the method: the casein of the milk studied is reversibly precipitated with an aqueous solution of 3M ammonium sulphate, then the precipitate is redissolved in a 0.85 per cent solution of common salt and restored to the original volume of milk; after that the casein content of the solution thus obtained is photometrically measured with the aid of the dye-binding (amido black 10 B) reaction of proteins. With their method the authors studied milk samples obtained from udder quarters of Schalm-negative and Schalm-positive reaction and found that in the case of animals with healthy udders the casein content relative to the total protein was 77—80 per cent or more. Milks with casein contents lower than 77 per cent are of abnormal composition. The method is recommended for use when accepting milks on the basis of their protein content and when testing milks in dairy plants.

Introduction

The opinion that milks used for industrial purposes should be accepted on the basis of their protein content becomes more and more general these days. It would be especially important in the field of cheese-, curd- and casein production. In the lack of a rapid and adequate method in the course of cheese production the protein content of the cheese milk too is adjusted on the basis of the total protein content (SZIGETI 1965). It is more appropriate, however, for both the dairy industry and the total national economy to determine the casein, because especially in industry-like large-scale cattle farming the physiological, pathophysiological, pathological and etiological factors, and conditions without which no high-quality milk production can be realized, must not be left out of consideration. If the milking machines, or their use, are not adequate, or else the hygiene of milking is neglected, then a mass occurrence of mastitis can be reckoned with in the dairy stocks. It is a fact based on pathophysiological and biochemical axioms that mastitis decreases the fat, casein, lactose and total mineral content of milk, while increasing its total protein content, and within this the whey protein- and chloride ion contents as well as the leucocyte count, and in general the activity of enzymes contained in it. This pathological change in the composition of milk is very often not conspicuous, especially in sub-clinical cases, although the great quantity of whey protein — in addition to impairing the quality — decreases the amount extracted from the low amount of casein available, especially in the production of hard and semi-hard cheeses (NYIREDI 1938).

Prior to discussing our investigation, we give here a survey of data concerning the milk composition of healthy animals (Table 1).

Table 1

Composition of cow's milk on the basis of a literary summary of various times and authors (g/100 ml)

Authors Data	B. MARTINY 1871	P. SOMMERFELD 1909	H. RIEVEL—O. FETTICK 1909	W. GRIMMER 1910	*B. BLEYER 1930	F. KIERMEIER 1968	Y. ANAGAMA 1972
Fat							
average	3.40	3.40	3.40	3.79	3.40	3.8	3.30
extreme values	—	—	—	—	—	5.4—6.1	—
Total protein							
average	3.20	3.30	3.50	3.41	3.50	3.3	2.84
extreme values	—	—	—	—	2.5—5.0	2.8—3.7	—
Casein							
average	—	3.00	3.00	2.81	3.00	2.5	2.22
extreme values	—		2—4.2	—	—	2.2—2.8	—
Percentage of casein to total protein							
average		90	85		85	80	78
extreme values	—	—	—	76—85	—	76—86	—

* cit GRIMMER et al.

Material and method

For the examinations milks from individual udder quarters were selected exclusively on the basis of the Schalm test (NYIREDY–MÓCSY 1965). Milks displaying a Schalm-positive reaction originated from cows suffering from sub-clinical mastitis. For casein determination the methods of Schlossmann—Hoppe-Seyler—van Slyke—Bosworth—Goetzl (cit. BÖMER 1936, GRIMMER *et* 1930, KIERMEIER 1968) are usually employed. Each method has the disadvantage of being *al.* linked with Kjeldahl's N-determination, and all of them require much time, space and work. They are thus unsuitable for a quick test performed in the dairy plant and for the reception of milk. The most rapid method is a photometric measuring based on the complex binding of amido black 10 B-protein with the Pro Milk II. automatic protein testing apparatus produced by the Danish firm A/S. N. Foss Electric, with which our Station has already performed total protein analyses (UZONYI 1971).

The instrument has the advantage that the results can be immediately read.

By the instrumental procedure casein can be determined with three different methods:

1. with an indirect method, namely, first the total protein is determined, and after the reversible or irreversible precipitation of the casein the protein content of the filtrate is measured and its value subtracted from the total protein content. The procedure is not practical, as the lower limit of measuring is 2.5 per cent which does not make the direct reading of results possible.

In case the casein content of the milk sample is below 2.5 per cent, the indirect method completed by some combined procedure can be used for casein determination in the following way: the total protein content of the milk sample examined is determined (*a*), then 10 ml of whey obtained from the milk sample, or the filtrate containing whey protein is mixed with 30 ml milk containing a known quantity of protein (c), then the total protein content of the mixture is determined again (b) and its casein content calculated by means of the following formula:

$$\text{casein content} = a - \frac{(10 + 30)b - 30c}{10}$$

(With the formula applied, the volume differences of whey or plasma extraction should — of course — be taken into consideration.)

2. The combined method employed by the firm A/S. N. Foss Electric is used in the following Way. First, the total protein content of the milk sample is determined (I), then the protein content of milk and distilled water mixed at a ratio of 1 : 1 (II). To a 100 ml quantity of milk heated to 40 °C 3 ml 33.3 per cent acetic acid is added and mixed, then after 10 minutes it is mixed again with 3 ml sodium acetate of n/3.33 and 3 minutes later filtrated. The protein content is also determined in the 1 : 1 ratio mixture of the milk sample and the filtrate obtained from it (III), and from the data thus obtained the casein content of the sample is calculated by the following formula:

I — (III — II + 0.03) = the casein content of The milk sample.

+0.03 is a correction determined by the firm in 47 comparative Kjeldahl tests.

3. The direct method elaborated at our Station is the following: the casein content of the milk sample is reversibly precipitated with the aqueous solution of ammonium sulphate of 3 M concentration. Precipitation is performed with a 1 : 1 ratio mixture of milk and ammonium sulphate; it is kept in a water bath for 5 minutes at 65 °C, then centrifuged for 5 minutes at 1000 r.p.m. After this the milk plasma is removed, the precipitate dissolved with 0.85 per cent aqueous salt solution in a water bath of 65 °C temperature; after some 5 minutes it is cooled to a temperature of 20°C and photometrically measured with the Pro Milk II apparatus.

It must be noted that in the case of milks with an SH° titre below 6.6 the casein sometimes does not fully precipitate, in this case the ammonium sulphate solution has to be acidified with acetic acid, but the acid content of the solution should not be more than one thousandth.

The complete precipitation of casein was controlled with paper electroforesis and the aluminous treatment of the milk plasma by Schlossmann's method.

Of the milk samples 10 ml was used for the milk tests, but the percentage deviation of the test with 10 and 100 ml milk used was also examined, with 11 replications with the following formula:

$$\text{deviation percentage} = \sqrt{\frac{\sum(\bar{X} - Xn^2)}{n}} \cdot 100$$

deviation percentage of a 10 ml milk sample: ±0.074%
deviation percentage of a 100 ml milk sample: ±0.002%;

thus the larger sample gives more precise results, though tests performed with 10 ml were also satisfactory.

A pilot examination of fats too was carried out with the Milko-tester II apparatus made by the same firm (Borsi—Varga 1971).

Results

If we take the measurement data of Table 2 into consideration, we find that the values of the casein are not sufficient by themselves for drawing conclusions on the extent to which the useful content of milk — and among others its casein content — decreases under the influence of mastitis; at the most, the extreme valuess uggest some abnormities here and there. To draw the right conclusion, data of similar character must be adopted from the literature (Grimmer 1930) which is presented in Table 3.

When comparing the values of this table on the percentage casein content of total milk protein with the literary and our own data, we can draw the definite conclusion that the percentage proportion of the average casein content to the total protein shows a decreasing tendency in a direct ratio to

Table 2

Trends in the useful content of milk

Schalm reaction of milks from udder quarters Denomination of data	0	±	1+	2+	3+	Positive by the indicator test
Number of samples (n)	**11 (n_0)***	**10 ($n_{0.5}$)***	**22 (n_1)***	**22 (n_2)***	**24 (n_3)***	**25 (n_{i+})***
Fat						
average	3.65	4.38	3.65	3.93	3.82	3.64
extreme values	3.05—4.00	3.00—5.80	0.40—5.70	1.25—5.05	0 —5.70	2.50—5.70
Total protein						
average	3.65	3.88	3.77	3.79	3.96	3.65
extreme values	3.00—4.00	3.35—4.60	3.55—4.95	3.15—5.10	3.10—6.00	3.20—4.85
Casein						
average	2.95	2.91	2.05	2.39	2.61	2.43
extreme values	2.80—3.70	2.45—3.55	1.10—2.75	2.00—2.65	1.25—3.00	1.80—3.70
Percentage casein to total protein						
average	83 ($\overline{X}_0$)	74 ($\overline{X}_{0.5}$)	65 ($\overline{X}_1$)	62 ($\overline{X}_2$)	57 ($\overline{X}_3$)	55 ($\overline{X}_{i+}$)
extreme values	77—93	73—77	32—77	52—74	34—72	43—69

* $+n_0$ = Schalm negative
$n_{0.5}$ = Schalm ± doubtful
n_1 = Schalm-1+
n_2 = Schalm 2+
n_3 = Schalm 3+
n_{i+} = Indicator positive

Table 3

Trends in the useful content of milk

Denomination of data	Udder quarter	
	healthy	diseased
Fat	5.71	4.79
Total protein	4.98	4.93
Casein	3.87	3.07
Percentage casein to total protein	77	62

the intensity of the Schalm reaction, which means the disturbed secretion of the udder, and as such must be regarded as an important index both from diagnostical and dairy industry aspects.

(With the extreme values, naturally, it must be taken into account that e.g. the total protein content of colostrum with 3 per cent casein is 9.13 per cent, the casein content in milk of udder tuberculosis origin 9.20 per cent, in the case of 2.39 per cent whey protein (Grimmer 1910, Grimmer *et al.* 1930).

On the basis of Table 2 we had to establish the limit values between milks of negative and doubtful reaction, which we carried out by comparing the two mean values (Sváb 1967).

The course of our calculations was the following:

$$\bar{X}_{0+0.5} = \frac{\sum X_i}{n_0 + n_{0.5}} = 77\,\%$$

The sum of squares is:

$$SQ_{0+0.5} = X_i^2 - \frac{(\sum X_i)^2}{n_0 + n_{0.5}} = 650$$

The standard deviation:

$$S_{0+0.5} = \frac{SQ_{0+0.5}}{n_0 + n_{0.5} - 1} = 5.65$$

The standard error of the difference (S_d):

$$S_0^2 = \frac{SQ_0}{n_0 - 1} = 19$$

$$S_{0.5}^2 = \frac{SQ_{0.5}}{n_{0.5} - 1} = 4.98$$

$$S_d = \sqrt{\frac{S_0^2}{n_0} + \frac{S_{0.5}^2}{n_{0.5}}} = 4.89$$

$$t = \frac{\bar{X}_0 - \bar{X}_{0.5}}{S_d} = 1.84$$

Degrees of freedom:

$$FG = n_0 + n_{0 \cdot 5} - 1 = 20$$

$$P = 10\%$$

Standard error:

$$S_{\bar{X}_{0+0.5}} = \sqrt{\frac{SQ_{0+0.5}}{n_{0+0.5}(n_{0+0.5} - 1)}} = \pm 1.24$$

Confidence limits:

$$h_1 = \bar{X}_{0+0.5} \quad t_{p10\%} \cdot S_{\bar{X}_{0+0.5}} = 79.28$$

$$h_2 = \bar{X}_{0+0.5} - t_{p10\%} \cdot S_{\bar{X}_{0+0.5}} = 74.72$$

$$t_{p10\%} \cdot S_{\bar{X}_{0+0.5}} \approx h \approx \pm 3$$

The number of samples necessary for the given confidence limits and approaching $\bar{X}_0$ and necessary in relation to the value $\bar{X}_{0.5}$

$$\frac{t_{p10\%} \cdot S_{0+0.5}^2}{h^2} = 21 = n_0 + n_{0.5}$$

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PECTINESTERASE ACTIVITY OF FIVE IRAQI APPLES AT DIFFERENT STAGES OF MATURITY

By

H. A. AL-JASIM, S. H. ALI

DEPARTMENT OF FOOD AND DAIRY TECHNOLOGY, COLLEGE OF AGRICULTURE,
UNIVERSITY OF BAGHDAD, ABU-GHRAIB, IRAQ

Changes in the activity of pectinesterase (PE) in five Iraqi apple varieties at different stages of maturity were followed. The enzyme activity was determined by titrating the carboxylic group released from the hydrolysis of pectin with dilute NaOH. The results obtained show that there was an increase in PE activity in these varieties with the progress of maturity. The maximum enzyme activities occurred when the fruit was fully ripe and ready for commercial harvesting. This increase in the enzyme activity was then followed by a general decrease, as the fruit matured and ripened on the tree. The data also indicate that there was a qualitative difference in the behaviour of these varieties.

Introduction

Pectic enzymes influence the consistency of fruit products because of their ability to hydrolyze pectic substances. Enzymes included in this group are: pectinesterase (PE), polygalacturonase (PG) and the pectin transeliminase. Pectinesterase hydrolyses pectin producing pectic or pectinic acid with the liberation of equivalent amount of methanol.

Many investigators studied the changes in the activity of pectinesterase in many fruits during ripening. NAGEL–PATTERSON (1967) demonstrated the increase of PE activity per pear during maturation and the decrease of this activity per gram fresh weight and per milligram protein. In tomato, DENNISON *et al.* (1954) found that PE activity was low until the fruits reached the mature green stage, at which time the activity rose sharply, until about two days after the fruits had begun to turn colour.

JACQUIN (1955) reported an increase of PE activity in apples and pears during ripening. WEURMAN (1954) demonstrated that PE activity was high in the young fruits of Doyenne Boussoch pears and then started to decrease rapidly, reaching a low level some two weeks before commercial picking time.

HULTIN–LEVIN (1961) reported the presence of three pectinmethylesterase fractions in banana and that the activity of all these fractions increased, as the banana skin began to change from green to yellow in colour.

The present investigation was undertaken to determine the changes in the activity of PE in five Iraqi apple varieties at different stages of maturity.

Material and method

Sampling and analysis. Apple samples were obtained from Zaafaranyia Horticultural Experimental Station, Baghdad. Five varieties were studied, they are: Ajmy, Sharabi, Sukkari, Basri and Koofi.

Equal number of fruits were taken from each of seven trees used for sampling. At the early stage of development about 200 fruits were picked, while at the later stage about 30 fruits were used. The fruits were washed, dried and cut into small pieces. Fifty gram samples were blended for 3 minutes with 100 ml of 1.5 M NaCl solution. The pH of the slurry was adjusted to 7.5 using 0.1 N NaOH solution. The slurry was left in a refrigerator (2—3°C) for 90 minutes and filtered through No. 2 Whatman filter paper into a Buchner funnel, with a slight vacuum. Hundred ml portions of the filtrate were collected from each sample and used for the assay.

Assay of PE activity. PE activity was determined at 30 °C by titrating the free carboxyl groups produced on deesterification of pectin with 0.02 N NaOH solution. The reaction mixture consisted of 20 ml 1-per-cent (w/v) apple pectin (250 grade produced by BDH) and 5 ml of enzyme extract. The pH of the mixture was maintained within the range 7.0—7.5 by the use of a Beckman Zeromatic pH meter. Immediately after mixing the enzyme extract and the substrate, the pH was adjusted to 7.5 and then a measured volume of the dilute alkali was added to keep the pH within the mentioned level. The reaction mixture was continuously agitated by a magnetic stirrer. The reaction time was 10 minutes. The activity of the crude enzyme was expressed as COOH μ-equiv./min./g fresh fruit.

Results

PE activity for 5 apple varieties during ripening and maturation are shown in Table 1. Data in this table indicate that there was an increase in the enzyme activities during the early stages of development reaching a maximum value at the beginning of commercial picking. The activities then decreased with the progress of maturity. The maximum enzyme activities occurred when the fruit was fully ripe and ready for commercial harvesting (May 27). This increase in activity was the greatest in Koofi, followed by Sukkari, Basri and Ajmy.

These results are in agreement with those obtained by JACQUIN (1955) in apple and by NAGEL—PATTERSON (1967) in pears.

Table 1

Changes in PE activity of five Iraqi apple varieties at different stage of maturity

Date of sampling	Stage of ripening	PE activity COOH μ-equiv./min./g fresh apple for the variety				
		Ajmy	Sharabi	Basri	Koofi	Sukkari
April 16, 1970	Small green	0.34	0.5	0.21	0.5	0.01
April 26, 1970	Mature green	0.65	1.1	0.58	1.0	1.16
May 11, 1970	Beginning of ripening	1.01	1.5	0.72	1.08	—
May, 27, 1970	Commercial harvesting	1.66	2.8	1.66	4.5	3.03
June 3, 1970	Commercial harvesting	1.08	2.00	1.08	3.61	2.17
June 15, 1970	All harvested except Sharabi	—	1.2	—	—	—

It must be pointed out, however, that the highest activities reported herein are relative values due to the arbitrary selection of sampling data.

The decrease in activity after the commercial harvesting stage noted is also in agreement with the results of WEURMAN (1954) on tree ripened pears.

In banana, HULTIN—LEVIN (1961) indicated the presence of 3 fractions of pectinesterase and stated that each fraction was synthesized by a separate pathway or from conversion of inactive form to active form. Conversely, the various fractions may serve as precursors to others. It could be a change occurring in the protein itself.

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NATURAL PARTHENOCARPY IN PEAR VARIETIES

By

J. Nyéki

HORTICULTURAL RESEARCH INSTITUTE, BUDAPEST—BUDATÉTÉNY

The natural parthenocarpy of 37 pear varieties was studied in 1968, 1969 and 1970, each year with a different number of varieties. On the basis of the results obtained parthenocarpic fruit production was found to be primarily a genetically determined characteristic of the variety. Its manifestation is, however, influenced by ecological factors. The ratio of parthenocarpic fruit setting to the number of fruits ripened may change from year to year. According to our investigations the manifestation of parthenocarpy is positively influenced by high (20—25°C) temperatures prevailing at the time of the fruit setting. According to the extent of natural parthenocarpy the varieties can be classified into six groups. Parthenocarpically set mature fruits — either completely seedless, or containing sacs consisting of mere seed-coat — were obtained from 3.4 per cent of the flowers treated. In the varieties examined apomixis was not observed.

Introduction

One of the objectives in the modernization of pear production is to increase the reliability of yield and the productivity of the varieties. Productivity can either be increased by breeding methods or by selecting suitable pollen varieties and determining their optimal proportions and arrangement within the plantation. The yield reliability of the varieties is also increased, however — in addition to growing site and disease resistance — by natural and induced parthenocarpy. The inconsistent literary data — obtained under different ecological conditions — on pear varieties (Griggs—Iwakiri 1954, Griggs *et al.* 1957, Gorter—Visser 1958, Kobel 1954, Nagy 1960, Maliga 1960, Karnatz 1963, Chollet 1965, Westwood *et al.* 1968, etc.), while proving their natural parthenocarpy, made the study of further problems relative to the question justified.

Material and method

The experiment was carried out with trees of our variety collection grafted to wild pear- and "A" quince stocks planted in 1953, at the Érd-Elvira Station of the Horticultural Research Institute, in 1968, 1969 and 1970. Daily mean temperatures during flowering and fruit set are contained in Table 1.

For the purpose of the examinations the flowers were castrated at the white bud stage. The flowers were isolated with parchment paper bags of a size of 25 × 35 cm. With one bag an

average of 25—30 flowers (4—5 cymes) can be isolated. The isolators were fixed with labels with the data of the treatments written on them. 100—300 flowers per variety or more were treated on each occasion. Of the methods of castration radical castration was applied, as in the pilot experiments no significant difference in the percentage of mature fruits was found between the methods of anthera- and radical castration.

According to our data with the method of radical castration a worker is able to castrate 300—350 flowers per hour in the pear varieties. The castrated flowers were isolated again in order to exclude the possibility of accidental pollination.

Table 1

Trends of the averages of daily mean temperatures during the flowering- and fruit-set periods of pear varieties in the years of the experiment, Érd-Elvira

Year	Flowering time of the varieties according to observations on the dynamics of flowering	Averages of daily mean temperatures during flowering °C	Deviation from values obtained in 1968 °C
1968	15—22 April	14.85	±0.00
1969	28 April—2 May	22.10	+7.25
1970	24 April—5 May	19.32	+4.44

The isolators were placed at random in a number of trees in each variety, at the average height of the crowns by the respective quarter of the globe, in order to obtain average fruit-set values characteristic of the variety. (It is a well-known fact that the extent of fruit set is influenced by the co-ordination of the trees, the rate of inflorescence, the age of the productive part and the position of the flower in the cyme, etc.)

When flowering in a variety had ended — the pistils had become brown in the isolators — the isolators were removed, except for a small piece of them left on the shoots to make the evaluation of fruit set easier. The first-fruit set evaluation was carried out after the small fruits had dropped from the generative parts, that is, 10—14 days after the time of mass flowering (when 70 per cent of the total number of flowers in a tree are at a functional stage).

The second evaluation was carried out after fruit drop in June, and the third one at the picking stage of the fruits.

The number of carpels as well as that of full and empty seeds per carpel were counted in the mature fruits. No full seed (capable of germinating) was found in the fruits examined. Apomixis — as supposed by MANZÓ (1956) — could not be proved by our investigations to exist in the pear varieties. As to the seeds of parthenocarpically set fruits two groups can be distinguished. Some fruits were completely seedless, while others only contained sacs consisting of seed-coat, which became brown or black by the time of ripening.

Results

A total number of 37 varieties were included in the experiments with a different number of varieties each year (Tables 2 and 3). The relatively large number of varieties made the further detailed study of natural parthenocarpy, on one hand, and the classification of the varieties according to their fruit producing capacity on the other, possible.

In the course of the experiment a total number of 9166 flowers were treated, 3.4 per cent of which resulted in mature fruits on the average of all the years taken in consideration.

Table 2

Natural parthenocarpy in pear varieties in 1968, 1969 and 1970, Érd-Elvira

Variety	Year	Number of flowers treated	Percentage fruit set to the total number oi treated flowers		Percentage of fruits brought to maturity	Characterization of parthenocarpy in each variety on the basis of the percentage of fully developed fruits
			After early dropping of small fruits	After fruit drop in June		
1. Amanlis vaj	1969	99	12.1	12.1	0.0	—
2. Arabitka (G)	1969	81	35.8	35.8	34.6	year by year regular tendency to parthenocarpy
	1970	169	41.4	21.9	21.9	
3. Bosc kobak	1968	112	1.0	1.0	1.0	year by year irregular tendency
	1969	145	0.0	0.0	0.0	
	1970	124	45.2	4.8	4.8	
4. Clairgeau	1968	146	1.3	0.0	0.0	no tendency
	1969	108	19.4	13.9	0.0	
5. Clapp kedveltje	1969	197	0.0	0.0	0.0	irregular tendency
	1970	102	23.5	15.7	11.7	
6. Conference	1968	102	70.5	0.0	0.0	—
7. Decaisne Henrik	1969	86	0.0	0.0	0.0	no tendency
	1970	80	0.0	0.0	0.0	
8. Diel vaj	1969	144	0.0	0.0	0.0	irregular tendency
	1970	154	0.0	0.6	0.6	
9. Dupuit asszony	1969	99	9.1	2.0	0.0	irregular tendency
	1970	138	30.4	5.8	5.8	
10. Esperes bergamott	1970	183	50.2	6.5	6.0	—
11. Favrené asszony	1968	127	3.9	3.9	0.0	—
12. Giffard vaj	1970	213	8.3	8.3	8.3	—
13. General Osmanvill	1969	150	0.0	0.0	0.0	—
14. Hardenpont téli vaj	1968	125	2.4	1.6	1.6	
	1969	104	3.8	3.8	3.8	regular tendency
	1970	252	5.6	2.8	1.5	
15. Hardy vaj	1968	103	1.0	0.0	0.0	
	1969	140	0.0	0.0	0.0	no tendency
	1970	91	0.0	0.0	0.0	—
16. Jeanne d'Arc	1970	106	15.1	0.9	0.0	—
17. Jodoigne diadala	1969	122	1.6	1.6	0.0	—
18. Liche Gusztáv	1970	180	0.0	0.0	0.0	—
19. Levavaseur	1969	108	20.4	18.5	16.7	—
20. Liegel vaj	1969	154	3.9	3.9	2.6	—
21. Magyar kobak	1970	129	10.1	10.1	10.1	—
22. Melló bárónő	1970	113	0.0	0.0	0.0	—
23. Monchallard	1969	102	0.0	0.0	0.0	—
24. Nemes Krasszán	1968	111	13.5	1.8	1.8	
	1969	140	60.0	40.0	25.7	regular tendency
	1970	95	20.0	16.8	16.8	

Variety	Year	Number of flowers treated	Percentage fruit set to the total number of treated flowers		Percentage of fruits brought to maturity	Characterization of parthenocarpy in each variety on the basis of the percentage of fully developed fruits
			After early dropping of small fruits	After fruit drop in June		
25. Őszi körte	1970	179	0.0	0.0	0.0	—
26. Pap körte	1969	116	0.0	0.0	0.0	no tendency
27. Pringalle vaj	1969	100	36.0	30.0	0.0	regular tendency
	1970	167	3.0	0.6	0.6	
28. Pisztráng 2	1968	126	0.0	0.0	0.0	—
29. Pisztráng 3	1970	106	21.7	15.0	15.0	—
30. Révész Bálint	1970	114	14.9	13.2	13.2	—
31. Sámson Ármin	1970	102	0.0	0.0	0.0	—
32. Serres Olivér	1968	243	2.2	0.0	0.0	irregular tendency
	1969	188	13.6	9.1	2.3	
	1970	158	63.2	5.1	3.8	
33. Stössel tábornok	1970	158	0.0	0.0	0.0	—
34. Téli esperes	1968	119	2.5	1.7	1.7	regular tendency
	1969	142	22.3	13.2	9.9	
	1970	151	2.6	2.6	2.6	
35. Téli Vilmos	1969	148	23.0	12.1	4.1	—
36. Totlében tábornok	1969	105	5.7	0.0	0.0	irregular tendency
37. Vilmos körte	1968	121	0.0	0.0	0.0	
	1969	176	0.0	0.0	0.0	
	1970	1278	2.2	1.3	1.0	

I. Natural parthenocarpy in the pear varieties. Table 2 shows the natural parthenocarpy of each variety examined. The data of the table disclose that the natural parthenocarpic fruit-producing capacity is a genetically determined characteristic.

Table 3

Grouping of commercial pear varieties by the extent of their tendency to parthenocarpy, on the basis of fruit set percentage determined after fruit dropping in June (1968—1970, Érd-Elvira)

No tendency to parthenocarpy 0%	Slight tendency to parthenocarpy 0.1–1%	Low tendency to parthenocarpy 1.1–5%	Medium tendency to parthenocarpy 5.1–10%	High tendency to parthenocarpy 10.1–20%	Very high tendency to parthenocarpy over 20%
Hardy vaj	Diel vaj	Bosc kobak	Dupuit asszony	Clapp kedveltje	Arabitka (G)
Pap körte		Hardenpont téli vaj	Serres Olivér	Téli esperes	Nemes Kraszszán
		Vilmos körte			Pringalle vaj

Of the varieties examined the following ones displayed no parthenocarpic fruit producing tendency: Hardy vaj, Pap körte, Decaisne Henrik.

Certain varieties, while setting a very high percentage of parthenocarpic fruits, do not bring them to maturity; abscission occurs either in June or before ripening (Amanlis vaj, Clairgeau, Conference, Favrené asszony, Jeanne d'Arc, Jodoigne diadala).

In some varieties, on the other hand, a high variability as to the extent of parthenocarpic fruit set occurs under the influence of temperatures prevailing at the time of flowering and fruit setting, e.g.: Clapp kedveltje, Pringalle vaj, Téli esperes.

In other varieties (e.g. Arabitka [G], Nemes Krasszán) parthenocarpic fruit set may be a domineering and stable characteristic of the variety on which climatic factors have little influence.

II. Extent of a natural tendency to parthenocarpy in the pear varieties. On the basis of literary references Table 5 summarizes the results obtained by other authors concerning natural parthenocarpy in the commercial varieties examined in our experiment, thus making a comparison with our own results also possible.

On the basis of the extent of fruit set determined after fruit dropping in June, we classified the varieties into the following six groups: 1. varieties showing no tendency to parthenocarpy (0 per cent); 2. varieties showing a slight tendency to parthenocarpy (0.1 —1 per cent); 3. varieties showing a low tendency to parthenocarpy (1.1 —5 per cent); 4. varieties showing a medium tendency to parthenocarpy (5.1 —10 per cent); 5. varieties showing a high tendency to parthenocarpy (10.1 —20 per cent); 6. varieties showing a very high tendency to parthenocarpy (over 20 per cent). The above classification of commercial varieties is presented in Table 3.

The relationship between the extent of parthenocarpy and the tem-

Table 4

Changes in the natural capacity of parthenocarpic fruit production in pear varieties, in 1968, 1969 and 1970, Érd-Elvira

Year	Number of varieties examined	Number of flowers treated	Percentage proportion of fruit set to the total number of treated flowers		Percentage of fruits brought to maturity
			After the early dropping of small fruits	After fruit dropping in June	
1968	12	1.435	8.3	0.5	0.5
1969	23	3.054	9.6	8.4	5.1
1970	25	4.677	13.5	5.1	4.8
Average of the years examined			10.4	4.6	3.4
Total number of flowers treated		9.166			

Table 5

Natural tendency to parthenocarpy in commercial pear varieties according to literary data (Nyéki, 1970)

Variety	Literary data confirming parthenocarpy			Practically no tendency to parthenocarpy
	low tendency	medium tendency	high tendency	
1. Clapp kedveltje		Boikoff (1942), Gorter—Visser (1958), Karnatz (1960), Blaja (1962)		Ewert (1909), Kamlah (1928), Schanderl (1932, 1938), Kobel *et al.* (1939), Manzo (1956)
2. Vilmos körte	Kobel (1954), Nagy (1960), Botez *et al.* (1960), Friedrich (1961)	Kamlah (1928), Boikoff (1942), Griggs—Iwakiri (1954), Karnatz (1960), Blaja (1962), Kolesnyikov (1962)	Batjer *et al.* (1967)	Ewert (1909), Schanderl (1932, 1938), Rudloff—Peichl (1953)
3. Hardy vajkörte	Kobel (1954), Maliga (1960), Friedrich (1961)	Griggs—Vansell (1949), Rudloff—Peichl (1953), Karnatz (1960)	Thile (1954)	Schanderl (1932, 1938)
4. Diel vajkörte	Kamlah (1928), Blaja (1962)	Kobel *et al.* (1939), Kobel (1954), Karnatz (1960), Friedrich (1961), Kolesnikov (1962)		
5. Bosc kobakja	Karnatz (1960)	Kobel *et al.* (1939), Kobel (1954), Maliga (1960), Blaja (1962), Friedrich (1961)	Stephen (1958)	Kamlah (1928) Nagy (1960)
6. Pap körte	Maliga (1960), Blaja (1962)	Kobel *et al.* (1939), Manzo (1956), Kolesnikov (1962)	Botez *et al.* (1960), Karnatz (1960)	
7. Nemes Kraszszán		Kobel *et al.* (1939)		
8. Hardenpont téli vajkörte	Kamlah (1928), Maliga (1960), Karnatz (1960)	Kobel (1954), Manzo (1956), Friedrich (1961), Blaja (1962)		
9. Conference		Kobel *et al.* (1939), Gorter—Visser (1958)		
10. Serres Olivér		Kobel *et al.* (1939), Manzo (1956)		Nagy (1960), Botez *et al.* (1960)
11. Téli esperes		Kobel *et al.* (1939), Kobel (1954)		Nagy (1960), Botez *et al.* (1960)
12. Esperen bergamottja	Karnatz (1960), Botez *et al.* (1960)	Kobel *et al.* (1939)		

peratures prevailing at the time of flowering and fruit set is shown by the data of Table 4.

The data of the tables suggest that on the average of the varieties examined higher (20—25°C) temperatures during flowering and fruit set have a positive effect on the extent of parthenocarpic fruit production and amount (percentage) of fruits brought to maturity.

III. Studies on fruit quality and commodity properties. The index numbers of fruit shape show that fruits developed through natural parthenocarpy are more oblong and cylindrical than those produced by controlled cross pollination. In some varieties (e.g.: Arabitka [G], Hardenpont téli vaj, Serres Olivér, etc.) these characters were not significant.

According to the data on ripening dynamics, in the summer and autumn varieties fruits developed through parthenocarpy ripen 1—1.5 weeks later than combinations obtained from cross pollination. Our data on the delayed ripening of fruits are confirmed by the tentative measurements of fruit growth dynamics in 1969, according to which — on the basis of measurements made every five days during the early dropping of small fruits and at the time of picking maturity — the growth rate of the length and width of parthenocarpically set fruits was slower than of those in combinations obtained from artificial cross pollination.

Conclusions

From the experiments the following conclusions can be drawn:

1. Natural parthenocarpy is primarily a genetically determined characteristic of the variety, the manifestation of which is influenced by ecological factors (first of all, temperatures and sunshine hours during flowering and fruit set) — as confirmed by literary data and our own experimental results. In this respect the pear varieties can be characterized as follows: *a)* varieties with no or practically no tendency to natural parthenocarpy; *b)* varieties showing a tendency to natural parthenocarpy coupled, however, with the abscission of fruits in the periods of fruit dropping; *c)* varieties inclined irregularly and in different degrees to natural parthenocarpy, depending on the climatic factors of the respective year; *d)* varieties with regularly different or stable tendencies to natural parthenocarpy.

2. On the basis of the extent of their natural parthenocarpy pear varieties can be classified into six groups (varieties with no, a slight, a low, a medium, a high and a very high tendency to parthenocarpy, respectively).

3. The ratio between the capacity of parthenocarpic fruit production and the number (percentage) of fruits brought to maturity may change from year to year; these characteristics of the varieties are positively influenced by

high (20—25°C) temperatures prevailing at the time of the fruit set. In this respect there are stable varieties independent of the climate, and unstable ones highly dependent on the climate.

4. According to our own experimental results (Nyéki 1970) and the literary data (Kobel 1954), in pear varieties with a medium inflorescence fruits brought to maturity from 3—5 per cent of the total number of flowers in the tree are considered to be a satisfactory yield. Our investigations suggest that a higher percentage fruit set and fruits brought to maturity can be attained through parthenocarpy.

5. Natural parthenocarpy contributes to the increase of productivity and yield reliability in pear varieties. Parthenocarpy is especially favourable in varieties belonging to the last two groups in cases when cross pollination between the varieties is hardly — or not at all — ensured.

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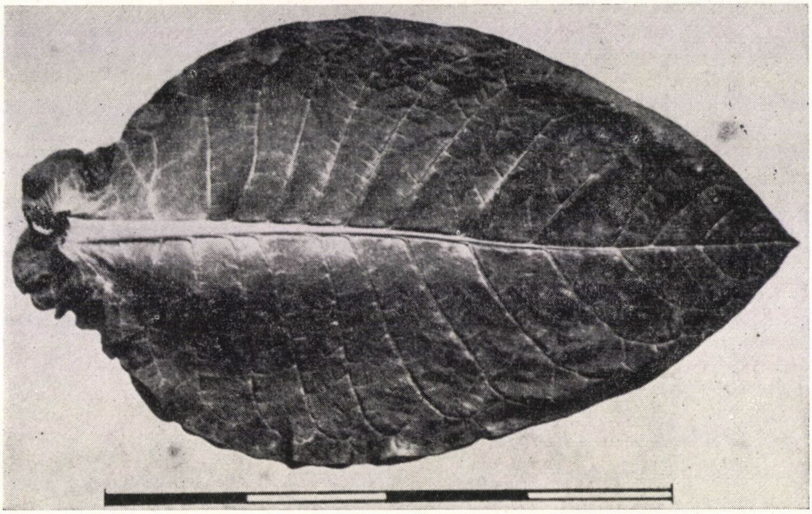
VARIA



TOBACCO "DEBRECENI"

Taxonomic place: Nicotiana tabacum L. var. *havanensis* Comes (DANERT 1961)

Origin: an old Hungarian variety of unknown origin; the earliest established local variety developed in the wide district of Debrecen*.

State qualification: variety licensed for circulation 1956; state- certified variety 1968.

General characterization: this rather resistant, high-yielding, drought- tolerant variety has long been a pipe tobacco much sought after, recently it has been used for poor-quality cigarettes too.

Morphological description:

Root system: of intensive growth, penetrating deep into the soil.

Shoot system: 110—160 cm high, conic barrel-shaped, thinly branching plant.

Stem: strong, yellowish green, consists of 20—21 nodes; some 2.5 cm thick at the base.

Foliage: the number of leaves can be 20—21 of which, however, only 10—14 are useful. The leaves are egg-shaped with pointed tips and a wide auriculate base half surrounding the stem; the leaf blade has an average length of 40—60 cm, and width of 20—35 cm; the veins are fleshy and join the main rib at an angle of about 70—74 degrees. The surface of the leaf blade is smooth, the leaves are thick and of coarse texture.

Inflorescence: a thick set polychasium of 160—180 cm length containing an average of 166 flowers.

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* Town in the eastern part of Hungary

Flowers: large, with an average length of 60 mm. The limb is about 30 mm in diameter, dark pink, slightly undulate, with poorly developed lobes.

Fruit: pointed-conic capsule, of brown colour and about 20 mm length, deeply dehiscent. The polychasium ripens 130—140 capsules.

Seed: dark brown; thousand-grain-weight 8.3 centigramme.

Biological characters:

Germination: the cardinal points are: minimum 15 °C, optimum 27 °C, maximum 32 °C; when germinating, the seeds absorb 177 per cent water in 12 hours (MÁNDY 1953.)

Development: medium rapid

Vegetation period: 90—130 days (there are more than one type); flowering starts at the beginning of July at the earliest, but may be postponed even to 25th July.

Water requirement: at an early stage of development requires much water, with this provided gives a high yield.

Resistance to diseases: not susceptible to diseases

Farm technology requirements:

Seeding: to hot-bed towards the end of March

Soil: prefers a medium heavy loam rich in nutrients, gives a high yield in soils of this type. (KAPÁS *et al.* 1965).

Productivity: leaf yield ranges from 14 to 16 q/ha; if the conditions are favourable it may even be more than that.

Growing district: it is grown in Hungary in the region east of the river Tisza, in the counties Hajdú-Bihar and Békés (KAPÁS *et al.* 1965).

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Prepared at the Department of Botany, University of Agricultural Sciences, Debrecen.

GY. MÁNDY

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DECOMPOSITION OF SOME ORGANIC MANURES AND THEIR EFFECTS ON NON-SYMBIOTIC NITROGEN FIXATION IN EGYPTIAN SOILS

Several investigators pointed to the importance of non-symbiotic nitrogen fixation in Egyptian soils, ISHAC (1958), ABDEL-HAFEZ (1962) and IBRAHIM (1964). Non-symbiotic nitrogen-fixing organisms were found to be present in high densities and this may be taken as a criterion of the amount of fixed nitrogen (JENSEN 1954).

These organisms, however, are well-known to be affected by the amount and chemical composition of organic matter amendments. FEDEROV—HERNANDES (1955), SMYK—MILKOUSKA (1957), EL-HADIDY (1960) and TAHA *et al.* (1965) found that organic manures, such as dung, compost, green manures, stimulated the growth and proliferation of non-symbiotic nitrogen fixers more than inorganic fertilizers.

The type of soil also affects the density of non-symbiotic nitrogen-fixing organisms, KELLY (1954), POCHON—BARJAE—LAJUDIC (1957), EL-SAID (1963), MAHMOUD—ABOUL-FADL—ELMOFTY (1964) and TAHA—MAHMOUD—IBRAHIM (1964, 1965).

The current investigation reveals the effect of the commonly applied organic manures on nitrogen fixation process in a fertile loamy soil and a newly reclaimed sandy soil of the Tahrir province.

Two types of soils were used in the present investigation: i, fertile clay-loam soil from the Experimental Station of the Botanical Section, Ministry of Agriculture at Giza. ii, sandy soil obtained from Tahrir Province which has been under reclamation since 1953.

The soils were air- dried, ground and sieved, then portions of 1 kg of each soil were placed in cylindrical plastic pots. Each soil was amended with the following organic manures: farm-yard manure, compost, city garbage and cotton- seed cake (Table 1).

Table 1

Chemical analysis of the organic manures used

Organic manure	Moisture %	Org. m. %	Total m. %	C/N	P_2O_5 %
Farm-yard	21.89	15.71	0.820	11.11	0.96
Compost	51.16	30.01	0.971	17.94	0.86
City garbage	18.88	18.42	0.680	15.75	0.64
Cotton-seed cake	7.06	66.25	3.510	10.95	1.99

Organic manures were added on the organic matter basis. This was carried out by supplementing the soil with 1 per cent organic matter, to give a fair picture for comparing their rates of decomposition and effect on nitrogen- fixation process. On this basis, the amounts of added organic manures were found to be 77.60, 50.36, 63.56 and 16-17 g per pot for the farm-yard manure, compost, city garbage and cotton- seed cake, respectively.

All the pots were kept under laboratory conditions, and the moisture content was continuously adjusted with tap water to 60 per cent of the water-holding capacity. Samples were drawn for chemical and microbiological analyses at 2 weeks' intervals for 10 weeks.

Chemical analysis. Organic matter. In manures: it was done by loss on ignition in an oven at 700 °C for one hour. In soil: it was determined according to Walkley and Black's wet digestion method (JACKSON 1958). Total nitrogen: the Kjeldahl method was followed according to JACKSON (1958).

Bacteriological determinations. Total microbial flora: by plating on soil extract yeast agar medium (MAHMOUD 1955). Non-symbiotic nitrogen fixing bacteria. *Azotobacter* was determined on base medium 77, and *Clostridia* on Winogradsky's medium after ALLEN (1961), using the dilution-frequency method.

Organic matter. The initial organic matter content of the loamy soil (1.81 per cent) was significantly higher than that of the sandy soil (0.372 per cent). Organic matter content showed a significant gradual decrease as the experiment proceeded. This could be attributed to the activities of soil microflora which resulted in the degradation of the organic matter. The rate of organic matter decomposition appears to be higher in sandy than in loamy soil.

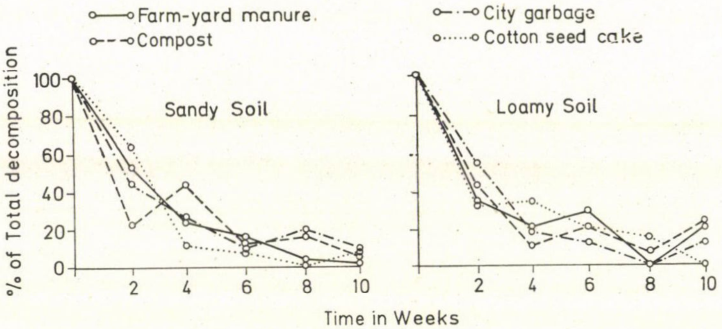

Fig. 1. Rate of organic matter decomposition

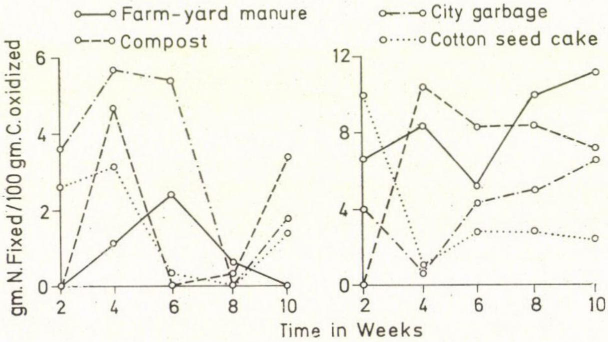

Fig. 2. Amounts of nitrogen fixed per 100 g of carbon oxidized

Percentage loss at the end of the experiment was found to be 25.51, 31.84, 34.55, 26.24 and 44.23, 42.90, 46.26, 46.72 for farm-yard manure, compost, city garbage and cotton- seed cake in the loamy and sandy soil, respectively This could be due to more aeration in sandy soile where soil particles are relatively large as compared to loamy soil. In general, the rate of organic matter decomposition was higher early in the experimental period. Especially in sandy soil (Fig. 1). This could be attributed to the presence of easily available organic materials early after the application of manures.

Total nitrogen. Addition of organic manures variably increased the total nitrogen content of both soils. This is due to the variation in the amount of total nitrogen initially present and to the amount added of each. However, the initial total nitrogen content of the loamy soil (0.108%) was higher than that of the sandy one (0.017%).

In general, an increase in the total nitrogen was recorded in all treatments including the control. This could be attributed to nitrogen fixation exerted by non-symbiotic nitrogen-fixing organisms. The presence of available energy sources in the amended soils enables nitrogen fixers to grow, fixing atmospheric nitrogen in the form of microbial protein. This, increase however. coincided with the gradual decrease in the organic matter content of the soil, resulting in narrowing the C/N ratios of both soils. Fig. 2 shows clearly that the amounts of nitrogen fixed per 100 g of oxidized carbon are significantly higher in loamy than in sandy soil. This was found to be in the average of 8.33, 6.79, 4.15, 5.59 gm. and 0.81, 1.68, 3.52, 1.48 g nitrogen due to the application of farm-yard manure, compost, city garbage and cotton- seed cake in the loamy and sandy soils, respectively.

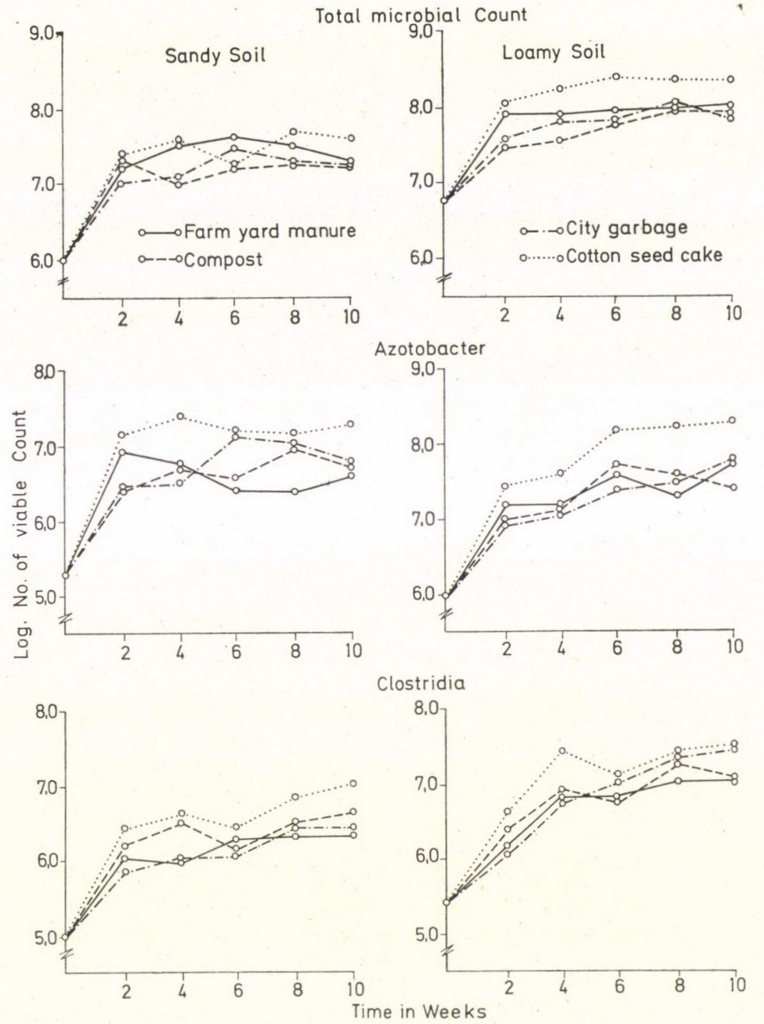

Fig. 3. Effect of organic manuring on total microbial Coynt and non-symbiotic nitrogen- fixing bacteria

It could also be seen that in spite of the relatively low amount of nitrogen fixed due to the application of cotton- seed cake, counts of nitrogen fixers attained the highest. This could be attributed to the presence of higher percentage of total and soluble nitrogen which induce more growth and proliferation of such organisms, being dependent on soil nitrogen rather than on atmospheric nitrogen. Therefore, the increase in nitrogen- fixers count could not be taken as index of nitrogen fixation in soils rich in nitrogen. Jensen (1954), however, stated that the extent of nitrogen fixers growth could be taken as an index of nitrogen fixation.

Total microbial count. The loamy soil initially contained higher microbial population than the sandy soil, being 5589 and 0.915 million/g dry wt. respectively. This is because loamy soil contains a relatively higher percentage of organic matter and inorganic nutrients than sandy soil which is newly reclaimed.

Organic manuring significantly increased the total microflora in both soils (Fig. 3), This observed increase could be attributed to the presence of easily decomposable organic matter and optimum moisture which favoured their growth and proliferation. In fact, the significant increase in soil microflora coincided whith the highest rate of organic matter degradation.

The marked response of total microbial flora to organic manuring which is higher in loamy than in sandy soil, shows that the physico-chemical properties of such soils are suitable for the proliferation of soil micro-organisms.

Cotton- seed cake showed also the highest microbial flora that was attained and persisted for a longer time in both soils. This could be attributed to the highest percentage of nitrogen in this manure (3.51 per cent). Nitrogen is well-known to be an essential element for micro-organisms to proliferate building up their microbial protein.

Non-symbiotic nitrogen- fixing bacteria

A) *Azotobacter.* The initial count of *Azotobacter* was found to be higher in the loamy than in the sandy soil, being in the average of 0.965 and 0.209 million / g dry wt. respectively. Organic manures greatly increased *Azotobacter* counts in both soils (Fig. 3). This increase was, however, significantly higher in the loamy than in the sandy soil, due to the physico-chemical properties of the soil. The peak of increase was found to be earlier in sandy than in loamy soil. This can be deduced to aeration which encouraged the rapid decomposition of organic matter early in sandy soil, supplying *Azotobachter* with available sources of energy, whilst it was relatively slower in the latter soil.

The highest counts of *Azotobacter* were generally obtained with cotton-seed cake. This was attributed to the presence of sufficient amount of nitrogen in such manure which encourage and support high densities of *Azotobacter*, depending on soil nitrogen rather than atmospheric nitrogen. On the other hand, the relatively low nitrogen content of the other manures induces *Azotobacter* to depend on atmospheric nitrogen showing a relatively lower counts. Confirming this suggestion, results of the chemical analysis showed that the amount of fixed nitrogen by a given amount of carbon was relatively lower in cotton-seed-cake treatment as compared with other manures. Therefore JENSEN's opinion (1954), as to take the growth of *Azotobacter* as an index of nitrogen fixation, is not valid in soils rich in nitrogen.

B) *Clostridia.* The initial count of *Clostridia* was found to be much lower than that of *Azotobacter*, being 241 and 88 thousand/g dry wt. of loamy and sandy soil, respectively. This, however, confirms results of earlier investigators who found that *Azotobacter* was found in higher densities than *Clostridia* in Egyptian soils, EL-HADIDY 1960, ABDEL-HAFEZ 1962, IBRAHIM 1964).

Clostridia significantly responded to organic manuring: a marked and gradual increase was generally recorded in their counts throughout the experimental period (Fig. 3). This could be attributed to the presence of easily decomposable organic matter, optimum moisture, and to the association between microbial population and *Clostridia.* The high microbial size observed as a result of organic manuring appears to deplete soil oxygen allowing *Clostridia* to proliferate.

It should also be mentioned that cotton-seed cake still supports higher densities of *Clostridia* than other manures, and this could be attributed to the presence of high percentage of nitrogen, as mentioned before with *Azotobacter.* ALEXANDER (1961) stated that micro-oganisms assimilating nitrogen had the ability to utilize ammonium and sometimes nitrate and other combined forms of nitrogen. In fact, high densities of both organisms were observed later, i.e., subsequent to ammonification and nitrification.

Organic manuring is of great importance in Egyptian soils. This can be attributed to the existence of factors enhancing organic matter degradation such as high temperature, which may reach 62° on soil surface (ABDEL-HAFEZ 1962), suitable moisture, physico-chemical properties of the soil . . . etc. This necessitates frequent applications of organic manures (ABDEL-HAFEZ 1962, IBRAHIM 1964).

Hence, it has been found of interest to study the decomposition rate of the commonly applied organic manures in Egypt, namely, farm-yard manure, compost, city garbage, and cotton- seed cake. Besides, their effect on non-symbiotic nitrogen fixers and nitrogen fixation has been investigated. These organisms were, however, found to be present in high densities in Egyptian soils, (ISHAC 1958, EL-HADIDY 1960, ABDEL-HAFEZ 1962, IBRAHIM 1964). This investigation was carried out in the two types of Egyptian soils, namely, the fertile clay loam, and sandy soil representing soils under reclamation.

It was found that the rate of organic matter degradation was generally high in both soils. This was deduced to the afore-mentioned factor explained above. The rate of degradation was significantly higher in the sandy than in the loamy soil. This could be attributed to aeration which enhanced the rapid decomposition of organic matter.

Total nitrogen showed a marked increase due to organic manuring. This could be attributed to the growth of non-symbiotic nitrogen fixers. The presence of available sources of energy in the amended manures enhanced such growth and proliferation. This suggestion is comfirmed bacteriologically since a high microbial size of *Azotobacter* and *Clostridia* was observed in the amended soils. The extent of nitrogen fixation was found to be higher in loamy than in sandy soil. The former soil proved to be amore suitable medium for nitrogen fixation than the latter.

Application of organic manures greatly increased the total microbial flora in both soils. This increase was found to coincide with the high rate of organic matter decomposition, and higher response was observed in the loamy than in the sandy soil. This could be attributed to the physico-chemical properties, available nutrient, manure composition, initial microbial size . . . etc. BRIGHT — CONN (1919), WAKSMAN (1927), VANDECAVEYE (1939), EL-HADIDY (1960) stated that the addition of various organic materials to the soil greatly stimulated microbial activity. The kind, nature and microbial activity are influenced by the nature and composition of the organic matter and by the inherited soil properties.

Similarly, the high densities of non-symbiotic nitrogen fixers, especially *Azotobacter*, stress their importance in nitrogen fixation in such soils, confirming earlier investigators. The addition of organic manures greatly stimulated their growth and activity in atmospheric nitrogen fixation, especially in the loamy soil.

Cotton-seed cake, however, supported high densities of nitrogen- fixing bacteria. This could be attributed to the presence of high percentage of nitrogen in this manure. These organisms will seek the easiest way, depending on soil nitrogen rather than fixing atmospheric nitrogen, as confirmed chemically. ALEXANDER (1961) stated that ammonium salts availed preferentially and often at a greater rate than N_2 from the atmosphere. Ammonia, liberated as a result of the ammonification process, is most effective in the inhibiton of nitrogen fixation, that is, the bacteria uses up nitrogen salt rather than N_2 from the atmosphere. In the present investigation high densities of these organisms were observed later when sufficient amount of NH_3—N and NO_3—N accumulated as a result of ammonification and nitrification processes. Such results were, however, confirmed chemically, NH_3—N and NO_3—N were found to increase as a result of the mineralization of soil organic nitrogen.

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Prepared at the Department of Microbiology. Faculty of Agriculture, Ain Shams University; Department of Microbiology, Faculty of Agriculture, Alazhar University, Cairo.

S. A. Z. MAHMOUD, S. M. TAHA, A. N. IBRAHIM

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HISTOLOGICAL CHANGES AND ACCUMULATION OF LIGNIN IN THE DEVELOPING ENDOCARP OF CHERRY

The most interesting part of stone fruits is the putamen whose wall is formed by the endocarp of the fruit. The gross histological changes of the fruit and the general histological aspects of fruit development have been dealt with by many authors. Within the subject the double origin of the endocarp tissue and the accumulation of lignin in the cell wall, which causes considerable tissue hardening, are very interesting. Studies on the histogenesis of the endocarp may provide a sound theoretical basis for breeding new varieties, paper-shell stone fruits.

The tissue components of the putamen have long been studied. The histological aspects of the fruit development were investigated by MILLARDET (1866), TUKEY—YOUNG (1939) in sour cherry, by STERLING (1953) in plum, by REEVE (1954) in *Rubus strigosus*, by ANTONI

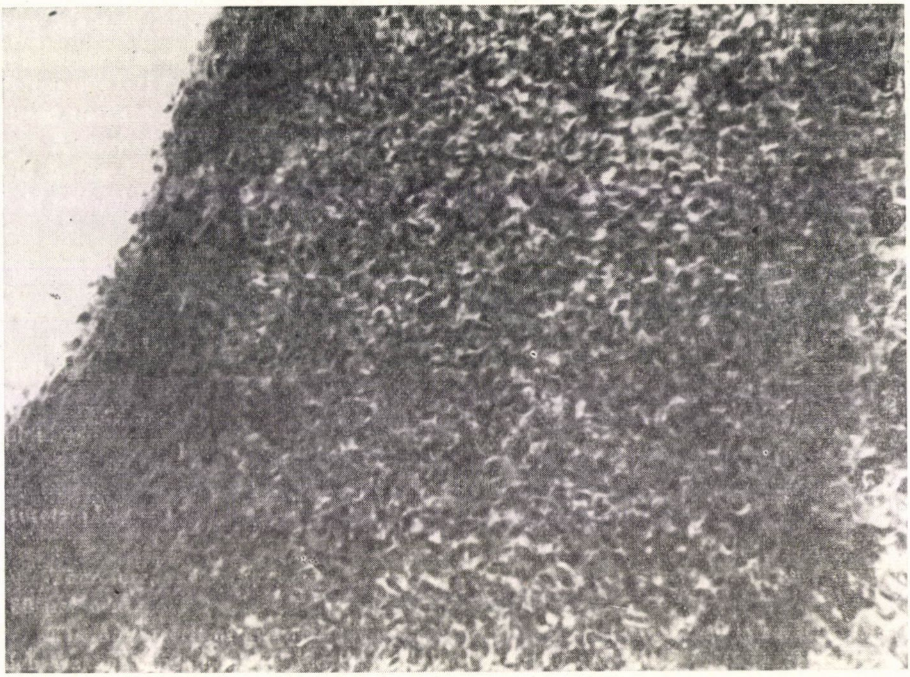

Fig. 1. Meristemic endocarp of the developing fruit of cherry on 14 April (40 ×)

(1971) in almond. PÉNZES (1957) gives an overall picture of the structure of the endocarp in *Prunus* species. According to the investigations, the endocarp tissue can be divided into two different tissue zones. Beside the inner epidermis, oblong macrosclereids develop (the authors think they derive from the inner epidermis), while the outer tissue zone of the endocarp (the part adjacent to the mesocarp) consists of isodiametric sclereids (considered to originate from the mesophyllum). Lignification in the endocarp of two peach varieties was examined by RYUGO (1962).

In the course of our investigations we collected cherry fruits of the Germersdorfi variety (*Cerasus avium* Mönch) from April to the beginning of June (from flowering to fruit ripening). Microscopic sections stained by microtechnical procedures, while from the more intensively lignified endocarp thin microscopic slides were prepared and studied with a Zeiss microscope. The chemical analysis of the lignin content of the endocarp was performed according to KLASON (1908) with a 72-per-cent sulphuric acid treatment, and as a result, a condensation product of native lignin obtained.

The pistil wall of the cherry flower (4 April) is 30—35 cell rows wide. The outer tissue zone of the mesophyllum contains meristemic cells situated isle-like among the permanent cells. The inner tissue zone of the mesophyllum is, on the other hand, of meristemic character all over (10—13 cell rows). This meristemic tissue zone is of a heterogeneous structure even at this stage of development: near by the inner epidermis it consists of tangentially flattened cells, while next to the mesophyllic tissue zone containing cells becoming permanent, of isodiametric cells (Fig. 1).

Cell division and cell elongation considerably increase the endocarp tissue, and by the end of the month (27 April) there are signs of cells becoming permanent in the endocarp tissue; the lignification of the putamen expressed by a tissue hardening begins at the apex. In the inner zone of the endocarp some 10 cell rows contain elongated cells (120—190 microns in length and 15 microns in width) with 1.2—1.5 microns thick cell-walls just beginning to become macrosclereids. Cells in the outer tissue zone of the endocarp too transform slowly into sclereids, but the thickness of their cell-walls is only 0.3—0.6 micron.

On 2 May lignification of the endocarp is expressed in the basal part too; the lignin content of the endocarp is 16 per cent of the dry weight. In the cells of tissues becoming sclerenchymatic there is only a thin plasm lining within the cell-walls. In spite of the lignification of the endocarp having started, there are still dividing cells found on the border of endocarp and mesocarp which further increase the endocarpic tissue.

6 May: the lignin content of the endocarp is 19 per cent. The walls of the macrosclereids are 1.5—3 micron thick. Adjacent to the mesocarp 30—35 cell rows of brachyscelereids are found with a diameter of 15—45 microns and cell-walls of about 1.5 micron in thickness. The more intensive thickening of the walls of the macrosclereids suggests a radial lignification. The cytoplasmic lining remains for a while in the vacuoles of the sclereids, and the plasmic contact- ensuring material transport between the cells is maintained through the canaliculate cell-walls.

In the subsequent weeks, due to an intensive consolidation, besides the thickening of the cell-walls, an increase of the lignin content can be observed in the endocarpic tissue;

	lignin content
12 May	35%
20	42%
25	46%
30	49%
10 June	54%

Changes in the lignin content of the endocarp are shown in Fig. 2.

On 10 June the wall of the macrosclereids in the endocarp of the fully ripened cherry is 4.5—8.5 microns thick. It is interesting that in the inner part of the tissue zone consisting of brachysclereids (adjacent to the macrosclereid tissue zone) the diameter of the sclereids is larger (30—57 microns), while the thickness of cell-walls smaller (2.4—3 microns) than in the tissue zone adjacent to the mesocarp (21—45 and 6—10.5 microns, respectively). In spite of this, according, to the calculations, the volume of the cell-walls is roughly the same, that is, they can contain identical amount of substances. The high degree of cell-wall thickening indicates that the plasmic contact between the cells is maintained for a long time (Fig. 3).

The above investigations have revealed that the tissue components of the cherry endocarp can already be detected in the pistil of the flower in a meristemic form. Intensive lignification starts in basipetal and centrifugal directions in the endocarpic cell-walls around the beginning of the second phase of fruit development. The two tissue zones of the endocarp — though mechanically inseparable — are sharply differentiated. According to the chemical analyses performed parallel with the histological studies, the lignin content of the endocarp rises very high, and in fully ripe fruits forms 54 per cent of the dry weight (RYUGO 1962 found only 39 per cent in the peach endocarp).

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Prepared at the Research Institute for Medicinal Plants.

Z. RÁCZ, P. GRACZA

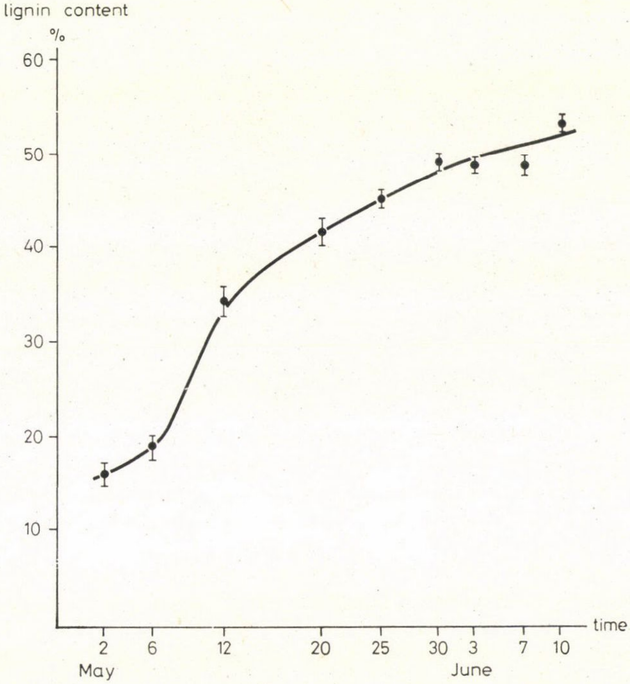

Fig. 2. Changes of lignin content in the cherry endocarp

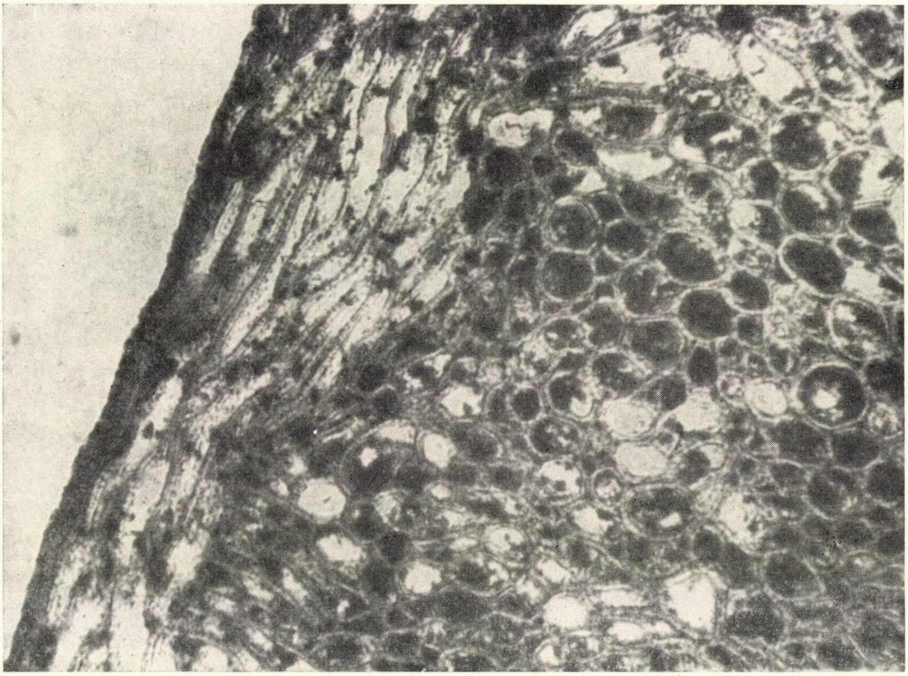

Fig. 3. Lignified endocarp in the fully developed fruit wall of cherry on 10 June (40 ×)

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EFFECT OF FOLIAR SPRAY WITH UREA AND/OR SUPERPHOSPHATE ON GROWTH AND DEVELOPMENT OF KENAF *HIBISCUS CANNABINUS* L.

The foliar nutrition of various plants has been the subject of extensive research. **Sibberstein—Writtwers** (1951), applying nutrient foliar spray on corn and tomato cultivated in green houses, found that various solutions increased the plant growth significantly, and similar results were obtained when lower concentrations were applied to the soil. **Hinsvark—Wittwer—Turkey** (1953) and **Boynton** (1954) reported that the treatment of apple with urea solutions of 0.75 per cent resulted in an increase in the level of the total amino and amide nitrogen. Urea also increased the chlorophyll contents of the leaves. **Webster—Varner—Gansa** (1955), using urea by foliar application on bean leaves, observed a higher amino acid and protein content in treated plants. **Boynton** (1954) demonstrated that several water-soluble phosphates containing P^{32} were promptly absorbed and translocated to other parts of the plants taking part in the processes of growth and development. **Mokhtar** (1968), studying the effect of foliar nutrition on flax, came to the conclusion that foliar application might not be efficient in plants with small area. **Kamel** (1969), studying the effect of foliar nutrition on sesame compared, with soil application, observed a significant increase in the yield of seed and the percentage of oil.

Studies on the effect of foliar nutrition with organic nitrogen compounds received little attention and hence the present study was carried out to investigate the effect of spraying with urea and superphosphate alone or mixed on kenaf.

The experiment was carried out in pots of 40 cm diameter in Giza Research Station during the 1968 season. Eight treatments, with five replicates were studied, each pot contained 2 kg of clean- washed sterilized sand. NPK fertilizers were added as follows: 7 g superphosphate, 5 g NH_4NO_3 and 4 g KCl before sowing.

Twenty-five seeds of kenaf (*Hibiscus cannabinus* L.) cv. Giza 3 were planted in each pot. After two weeks of germination, plants were thinned to 15 per pot.

The experiment began on May 26th and the plants were harvested on October 20th. Plants of each pot were sprayed with 50 ml of solutions containing urea or superphosphate alone or together with 0.5 per cent deterbon as a spreader.

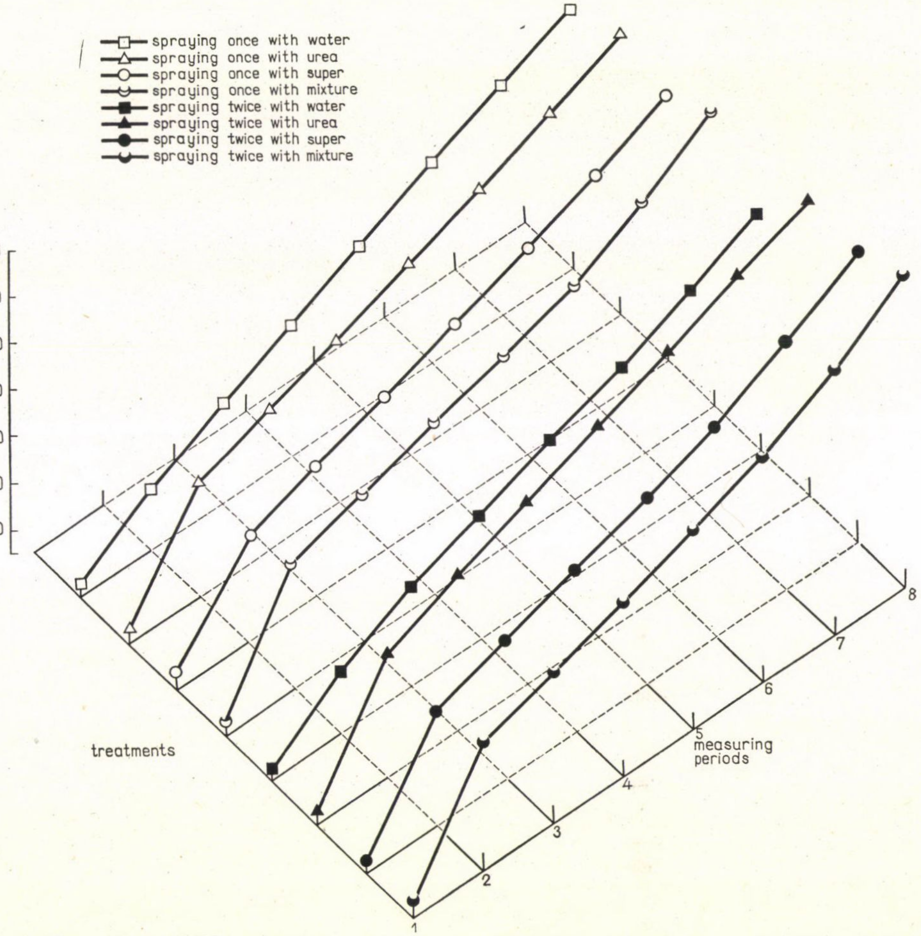

Fig. 1. The effect of foliar nutrition with urea and/or superphosphate on the plant height

Treatments were as follows. Spraying once at growing stage with dist. water (control), 1.2 per cent urea solution, 0.9 per cent superphosphate solution and with a mixture of 0.6 per cent urea and 0.45 per cent superphosphate solution. Spraying twice at growing and at the beginning of flowering stages with water (control), 1.2 per cent urea solution, 0.9 per cent superphosphate solution and with a mixture of 0.6 per cent urea and 0.45 per cent superphosphate solution.

The height of the plants, their weight and the final yield of fibre were measured and the data obtained were statistically analysed.

The plant length. Fig. 1 shows that spraying once with urea and/or superphosphate solution at the growing stage caused a significant increase in plant length. The increase was more prominent in the presence of the mixture and reached 45 per cent higher than the control.

The effect of superphosphate alone was of short duration, but the effect of urea alone or coupled with superphosphate continued throughout the experimental period.

Spraying twice at the beginning of the flowering stage, activated the elongation process particularly when the mixture was applied; an effect that continued till maturity. Plants of these treatments were 17 per cent taller than single-sprayed ones.

Table 1

The effect of foliar nutrition with urea and/or superphosphate on vegetative growth and flowering of kenaf

Treatments	Flowering date	Vegetative duration before flowering, in days	Flowering duration, in days
Spraying once with:			
dist. water (control)	15—8	81	35
Urea	22—8	88	37
Superphosphate	9—8	75	28
Urea+superphosphate mixture	12—8	78	22
Spraying twice with:			
dist. water (control)	15—8	81	36
Urea	25—8	91	40
Superphosphate	7—8	73	22
Urea + superphosphate mixture	12—8	78	25

Vegetative and flowering periods. Table 1 shows that spraying kenaf plants once at the growing stage with urea delayed flowering for 7 days, thus increasing the vegetative period for the same length of time. The flowering period was increased by 2 days compared with the control. However, spraying once with either superphosphate alone or with urea + superphosphate induced flowering 3 to 6 days earlier than the control, thus similarly shortening the vegetative period. Moreover, the flowering of such treated plants ended 13 to 16 days earlier than the control.

A second spray at the flowering stage with urea prolonged the duration of the flowering and vegetative stages by 3 days compared with the single spray. However, the second spray with superphosphate induced flowering 8 days earlier and shortened the flowering period too by 14 days compared with the control plants. The result of a second spray with the mixture was similar to the single-spray treatment with the same mixture.

The yield. Table 2 shows that spraying once at the growing stage with urea solution caused a significant increase in all the different components of yield, except the fibre weight. In the presence of superphosphate. urea caused a highly significant increase in the yield components too, with the exception of the fibre weight, which was increased only slightly. Superphosphate alone had no significant effect on the yield component, except the fibre weight which showed a significant increase.

The second spray at the beginning of the flowering stage with urea alone or coupled with superphosphate caused a highly significant increase in the different components of yield that was more remarkable in the presence of phosphate.

Spraying twice with superphosphate alone caused a slightly significant increase in the different components of the yield, however, the increase in fibre weight was highly significant reaching 84 per cent in comparison with the control.

Spraying with urea increased the plant height and its effect continued till the maturity stage. However, the superphosphate effect was observed for only one week after spraying and then disappeared. Spraying once with urea prolonged the vegetative period, increased

Table 2

The effect of foliar nutrition with urea and/or superphosphate on kenaf yield

Treatments	Weight of whole plant g	Weight of one plant without fruits g	Fruit weight of one plant g	Fibre weight of one plant g
Spraying once with:				
dist. water (control)	20.8	13.8	7.0	2.8
Urea	25.9	17.3	8.6	3.5
Superphosphate	23.5	15.9	7.6	4.4
Urea + superphosphate mixture	27.0*	17.9*	9.1*	3.5
Spraying twice with:				
dist. water (control)	21.4	14.0	7.4	2.6
Urea	27.6*	18.3*	9.3*	4.8
Superphosphate	24.8	16.9	8.9	4.8
Urea + superphosphate mixture	30.0*	19.7*	10.2*	5.0
L.S.D. 5%	3.92	2.88	1.44	1.02
L.S.D. 1%	5.32*	3.91*	1.95*	2.01*

the number of branches, delayed the flowering period and prolonged its duration, though a high amount of the photosynthetic compounds were exhausted in these processes. Accordingly, a significant increase took place in all the different components of the plant yield, except the fibre weight. However, spraying once with superphosphate shortened both the vegetative and the flowering periods, decreased the number of branches of the plant, and pushed the metabolic path towards fibre building, leading to the observed increase in fibre weight.

When urea was coupled with superphosphate, a highly significant increase in the different components of the plant yield except the fibre weight was noticed. This may be due to the low dose of phosphate which was masked by the effect of urea.

When the dose of superphosphate was increased by a second spray at the flowering stages, the effect of these two elements alone or mixed with each other, showed a highly significant increase in the fibre weight.

These results according to HINSVARK—WITTWER—TURKEY (1953), BOYNTON (1954), BURR *et al.* (1957) and RUBIN (1961) might be due to the stimulation of the chlorophyll synthesis and photosynthetic rate, that lead to the accumulation of photosynthetic products which are used later in the processes of growth and development.

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Prepared at the Physiology and Crop Nutrition Department, Ministry of Agriculture, Giza, Orman

R. FAHMY

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A METHOD OF SEED TREATMENT WITH VITAMIN SOLUTIONS FOR EATING PAPRIKA

The vitamins, as the regulators of the most important processes of metabolism, take part in the synthesis of amino acids, proteins, purine and pyrimidine bases and of nucleic acids. Their role in the activities of enzymes is also well- known; here they have definite functions in developing the properties. The protein parts of the enzymes enter into chemical interaction with the substrate in most cases together with an active group, the so-called coenzyme. Very often the active group of the enzyme is formed by some vitamin. According to their biological effect the vitamins belonging to group "B" are found in low quantities in the plant organism, their synthesis is ensured by the plant, though probably not in quantities sufficient for the adequate development of economically important characters. Adequate quantities and ratios of these vitamins, when ensured in the vitamin complex of plants may provide a possibility of increasing their productivity.

We relied on these elementary notions and theories when setting the aim of increasing the earliness, yield and value of the components of eating paprika.

The adequate quantity of vitamins was ensured for the plants by seed treatments. The experiments were carried out at the Horticultural Research Institute with the indeterminate variety "Javított Cecei" and the rosette type "Gépi konzerv". For the seed treatment vitamins belonging to group "B" — biotine, thiamine, pyridoxine, meso-inosite, pantotene acid, nicotine acid and cobalamine — were used in various concentrations and combinations. The seed treatment was carried out with a technique elaborated at our Institute and patented by the National Patent Office.

The experiments were laid out in a 4-replication Latin block design with 28 and 56 plants, respectively, per plot, sown in twin rows at a spacing of 50 × 20 × 20. The results were evaluated with variance analysis. This paper only presents the data of the three treatments showing the best results.

The effect of seed treatment with vitamin solutions was felt throughout the whole period of seedling raising. Seeds treated with solutions of meso-inosite, nicotine acid and cobalamine germinated 2—3 days earlier than the untreated seeds. Seedlings raised from treated seeds were more viable and thick-set and possessed well- developed root systems. Consequently, seedlings transplanted in the field were better established and the course of their development was accelerated as well. The earlier development and higher number of reproductive organs resulted in an earlier, larger and better quality yield.

Table 1 shows the results of experiments carried out with the eating paprika variety "Javított Cecei" in 1969 and 1970.

Table 1

Effect of seed treatment with vitamin solution before sowing on the earliness and yield of the eating paprika variety "Javított Cecei"

(Site of experiment: Horticultural Research Institute, Budatétény)

Time of picking	Control Yield q/cad. yoke	Meso-inosite			Nicotine acid		
		yield q/cad. yoke	Yield %	Surplus q/cad. yoke	Yield q/cad. yoke	Yield %	Surplus q/cad. yoke
In 1969							
I. 13 August	40.5	66.2	+63	+25.6	55.7	+37	+15.1
II. 27 August	27.7	26.2	— 5	— 1.6	23.3	—16	— 4.4
III. 24 September	46.7	50.8	+ 9	+ 4.1	46.2	— 1	— 0.5
Total picking	114.9	143.2	+25	+28.3	125.2	+ 9	+10.3

S.d.$_{1\%}$ for picking I = 17.6
S.d.$_{5\%}$ for picking I = 13.0
S.d.$_{5\%}$ for the total yield = 27.0

Time of picking	Control Yield q/cad. yoke	Meso-inosite yield q/cad. yoke	Yield %	Surplus q/cad. yoke	Nicotine acid Yield q/cad. yoke	Yield %	Surplus q/cad. yoke
In 1970							
I. 5 August	23.2	36.2	+56	+13.0	36.9	+60	+13.7
II. 18 August	58.2	67.3	+16	+ 9.1	62.7	+ 8	+ 4.5
III. 8 September	39.6	41.0	+ 3	+ 1.4	34.3	—13	— 5.3
IV. 6 October	48.5	42.7	—12	— 5.8	43.7	—10	— 4.8
Total yield	169.5	187.2	+11	+17.7	177.6	+ 7	+ 8.7

S.d. 1% for picking 1 = 10.6
S.d. 5% for the total yield = not significant

Earliness is well- illustrated by the yield surplus obtained on the first occasion of picking. When expressed in days, the first fruits can be picked 5—8 days earlier. In plots treated with meso-inosite the first- picking resulted in a 63–56 per cent yield surplus and, in addition, the amount of first-class commodity was twice as much as in the untreated plot. Seed treatment with nicotine- acid solution increased the berry weight by 37—60 per cent, and the proportion of the first- class commodity by 48—60 per cent compared to the control. In case the weight of the berries in the first picking attained 35—40 per cent of the total yield, the second picking resulted in a yield lower than that of the control (see the experiment in 1969). In the 1970 experiment the yield of the first picking did not exceed 20 per cent of the total yield, so even the second picking showed a significant yield difference. As regards the total yield, the difference was only significant in 1969 in the meso-inosite seed treatment.

Table 2

Effect of seed treatment with vitamin solution before sowing on the earliness and yield of the eating paprika variety "Gépi konzerv"

(Site of experiment: Horticultural Research Institute, Budatétény)

Time of picking	Control yield q/cad. yoke	Meso-inosite			Nicotine acid		
		Yield q/cad. yoke	Yield %	Surplus q/cad. yoke	Yield q/cad. yoke	Yield %	Surplus q/cad. yoke
1970							
I. 3 August	25.3	35.0	+38	+ 9.7	33.0	+30	+ 7.7
II.. 17 August	30.8	28.7	— 8	— 2.1	32.9	+ 6	+ 2.1
III. 9 September	13.0	12.2	— 6	— 0.8	21.4	+64	+ 8.4
Total yield	69.1	75.9	+ 9	+ 6.8	87.3	+26	+18.2

$S.d._{5\%}$ for picking I. = 8.8
$S.d._{5\%}$ for the total yield = 10,4

1971							
I. 20 July	15.5	27.5	+77	+12.0	23.4	+51	+ 7.9
II. 3 August	40.4	34.9	—14	— 5.5	37.7	— 7	— 2.7
III. 18 August	11.1	14.4	+29	+ 3.3	18.1	+45	+ 7.0
IV. 20 September	14.1	12.9	— 8	— 1.2	16.9	+20	+ 2.8
Total yield	81.1	89.7	+10	+ 8.6	96.3	+18	+15.2

$S.d._{5\%}$ for picking I. = 8.5
$S.d._{5\%}$ for the total yield = 12.5

Table 2 presents the yield results of experiments performed with the eating paprika variety "Gépi konzerv" in 1970—1971. In the meso-inosite treatment the first picking showed a 38—77 per cent yield increase, and the proportion of the first class commodity was 75—93 per cent higher than in the control. Cobalamine seed treatments resulted in no significant yield differences in either of the years at first picking, but the proportion of the first- class commodity was 75—125 per cent higher than in the control. As regards the total yield, on the other hand, a significant yield difference was found between the treated and untreated plots. From this we can draw the conclusion that the effect of cobalamine is felt throughout the whole vegetation period.

The difference in earliness between the two years of the experiment — as shown by Table 2 — is explained in the first place by the different time of the first picking. Both vitamins seem to have exercised a higher influence on earliness in 1971. This year, namely, the yield of the first picking only amounted to 20—30 per cent of the total yield, because it was carried out two weeks earlier than in the previous year when on the first occasion of picking 36—46 per cent of the total yield was already harvested. The first picking carried out relatively late in 1970 did not make a correct assessment of the effect of the vitamin treatment possible. Thus, in the case of the variety "Gépi konzerv" the effects of vitamins are more outstanding if the first picking is carried out earlier.

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Prepared at the Horticultural Research Institute, Budapest

M. SINKOVICS

FACTORS CONTROLLING ADVENTITIOUS ROOT INITIATION IN PHASEOLUS MUNGO (URD) HYPOCOTYLS

It has been established that in mung- bean hypocotyls, adventitious roots differentiate from the specialized phloem parenchyma cells, which, from a normally non-dividing stage, become meristematic (CHANDRA *et al.* 1971). The theory which has been documented so far, reveals that a complex and not clearly understood hormonal interaction controls the initiation of adventitious roots (DORE 1965). It has been reported that the exogenous application of I.A.A. (JACKSON—HARNEY 1970) and abscisic acid (CHIN *et al.* 1969) increase the root numbers two to three times, while kinetin (KAMINEK 1968) and G.A. (BRAIN *et al.* 1960) suppress root initiation. SKOOG—MILLER (1957), have demonstrated the role of hormones in organogenesis. Since there is no available information on the possible relationship between leaves, buds and adventitious root initiation in the hypocotyl, the present work has been performed.

Cuttings were prepared from one-week-old seedlings of *Phaseolus mungo* L. (urd), which were excised 3 cm below the cotyledonary node and dipped through the cut hypocotyl end in distilled water. Samples were prepared representing intact seedlings (+ leaves and bud), intact leaves (— buds), intact buds (— leaves) and decapitated leaves and bud. One set of each in fifteen replicates was placed under continuous white fluorescent light and the other under complete darkness at room temperature (21 ± 1 °C).

Only light- exposed seedlings responded to rooting, while the darkened ones failed to do so. The readings obtained (Fig. 1) indicate that the actual initiation of rooting in the two corners corresponding to vascular traces of primary leaves only started in intact seedlings first after 8 days. After 9 days additional rootings were intiated corresponding to the two vascular traces of cotyledons in intact seedlings and the two vascular traces of primary leaves in seedlings without buds. After 10 days, intact seedlings initiated adventitious roots above the first rooting zone, while the seedlings without buds initiated additional rootings corresponding to two contyledonary vascular traces. Samples without leaves also initiated the formation of two adventitious roots corresponding to the cotyledonary vascular traces and not to the primary leaf vascular traces. After 11 days both intact seedlings and seedlings without buds developed profuse adventitious roots forming one zone above the other, while samples without leaves remained undifferentiated in the zone of primary leaf vascular traces. The decapitated seedlings also initiated the adventitious root formation in the zone corresponding to the vascular traces of primary leaves. Furthermore, the adventitious root development only continued increasing in the two samples correponding to intact seedlings and seedlings without buds, while in others the position remained the same.

The data obtained explain the hypothesis that there are certain active ingredients, maybe hormonal in nature, the synthesis and translocation of which is mainly controlled by the primary leaves in light. Moreover, the path of translocation of these unknown factors is definitely through the vascular traces because of the fact that rooting is generally initiated in the corners corresponding to these traces. The presence of a bud definitely accelerates the rate of root initiation, possibly because of the substitution of some active ingredient (unknown) along with the leaf factor. It is difficult to assess their relationship which may be synergistic or additive. The discovery of four new peroxidase enzymes in the part of the cutting that produces roots (CHANDRA *et al.* 1971), which are in addition to, and different from, the three already present in the hypocotyl, suggests that these leaf and bud factors possibly initiate the synthesis of these isoenzymes, which might have been responsible for the differentiation.

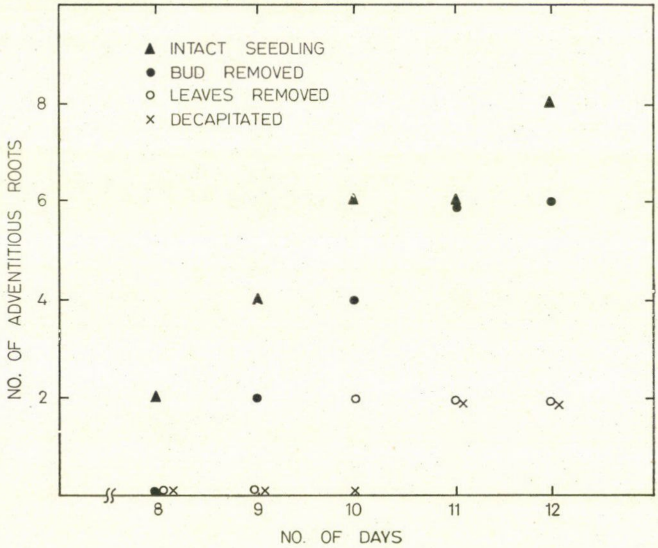

Fig. 1. Rooting intensity in mung- bean hypocotyl

The absence of rooting in dark in any situation may be interpreted as if the leaf and bud factors or the further synthesis of metabolites or enzymes, controlled by these factors responsible for differentiations, were inhibited. The work of KRUL (1968), that the uncoupler like 2, 4- dinitrophenol (DNP) promotes the formation of root primordia in the dark, suggests the possible involvement of the uncoupling of oxidative phosphorylation in root initiation. Furthermore, from the hypothesis that the other uncouplers and inhibitors did not enhance rooting, nor the ATP influenced the DNP effect, it seems possible that DNP might be acting indirectly to suppress the formation of repressing proteins to initiate rooting (BLACK—RICHARDS 1967). This was ruled out because of the fact that RNA- and protein- synthesis inhibitors were generally inhibiting or had no influence on root initiation (KRUL 1968). The work of KRUL (1968) suggests that IAA alone increases root initiation in the hypocotyl in dark and only higher concentrations of IAA with DNP become effective in increasing rooting. NDP as a root initiation factor was reported (KRUL 1968) to be more effective than IAA in dark but both became ineffective when the hypocotyl was transferred to light. He further suggested that IAA might have been involved in the metabolism of the endogenous root-promoting factor or acted independently as a triggering agent. The possibility which can be visualized from the above discussion is that light controls the synthesis of the endogenous

root- promoting factor or the translocation and activity of IAA as a triggering agent for the root initiation. The possible mechanism involved through the plant organs in initiating rooting -n the hypocotyl is still to be worked out and needs thorough investigation.

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Prepared at the Department of Botany, Punjab Agricultural University, Ludhian (Punjab), India.

A. K. Srivastava

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LIGHT- AND ELECTRON- MICROSCOPE STUDY ON THE EMBRYO SAC OF FIELD POPPY

The ovular structure, histogenetic conditions and seed development of field poppy (*Papaver rhoeas* L.), as studied by a light microscope, have been dealt with in a number of papers (Soueges 1936, Hasitschka 1956, Röder 1958, Paál—Gracza 1969). Only a few data are available (Israel 1964, Diboll 1968), on the other hand, on the electron, microscopic structure of the ovule in various plant species. And as to *Papaver rhoeas*, no observation has been made so far. The present paper describes the light- and electron- microscopic structure of the fully developed embryo sac of field poppy.

The material required for our examinations was collected at the stage of new-blown flower, and on the second and fifth day after blossoming, respectively. The light- microscope observations were made on section series fixed in Bouin solution and imbedded in paraffine, while the elctron-microscope examinations on ultra-thin sections fixed in 2 per cent buffered potassium-permanganate solution and imbedded in durcupane.

The ovule is bordered by two integuments. The outer integument is built up from two cell layers of which the outer layer consists of greatly enlarged cells in the process of stabilization. The cells of the inner layer, on the other hand, are still highly meristemic; it is from them that the crystalliferous cells will develop. The inner integument consists of three cell layers. The first layer is at an advanced, the third at an initial stage of stabilization. The middle cell

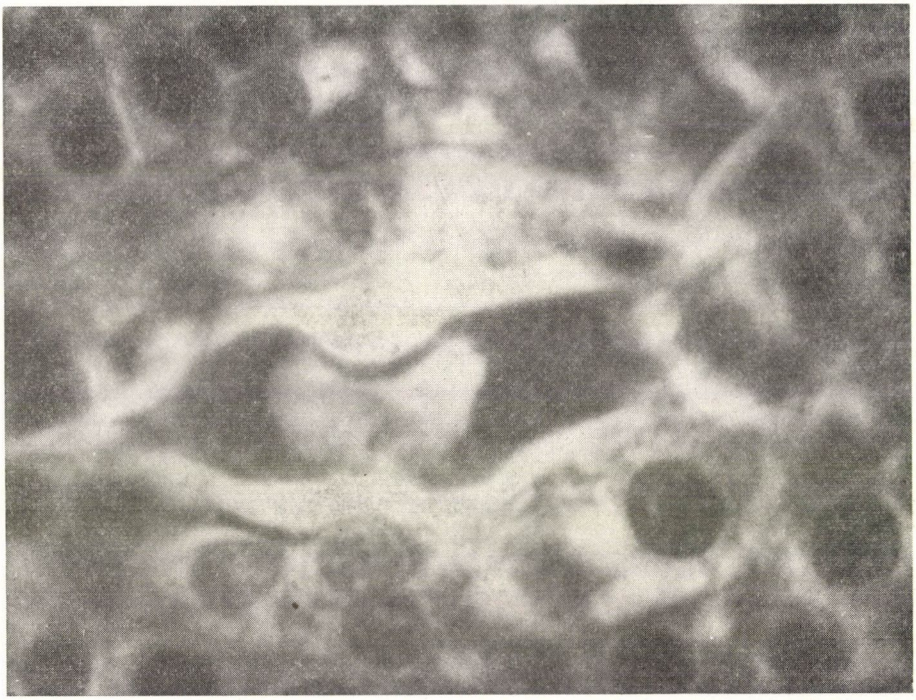

Fig. 1. Ovule of *Papaver rhoeas* with embryo sac, with hardly visible plasm bridges in the vacuole. (Obj. 40×, oc. 4×)

layer is still meristemic. In the chalaxal part the tissue of the nucellus is of a larger dimension, while in the micropylar zone — due to the growth of the embryo sac — remains but a few cell layers thick. The cells are stabilized, in the leucoplasts starch grains are separated. The eight-celled embryo sac is of a *Polygonum* type. The antipodes (three) are much larger than the central cells or those of the egg apparatus. Light- microscope examinations at a higher amplification reveal plasma filaments and bridges in the large vacuole of the embryo sac between the antipodes and the egg apparatus, which, however, seem to be structureless under such conditions of examination. In many cases the plasm precipitates during the microtechnical treatment and shows a thicker- lined structure, thus becoming visible. With adequate conditions of preparation, however, it is only in the central vacuole of the embryo sac, in the direction of the central cells, that some structure, suggesting the presence of plasm, can be observed (Fig. 1).

Observations made by an electron microscope revealed minute characteristics too in the vacuole of the embryo sac. This large vacuole — besides being filled with a milk-like cell-fluid — seems to be interwoven with a fine network of cytoplasm (Fig. 2). The fine threads of cytoplasm surround slightly elongated pentagonal or hexagonal spaces, and at the edges, on the border of the vacuole form a continuous plasm tube, on the one hand, on the other hand, a plasmic connection develops with the cells surrounding the embryo sac, and between the eight cells of the embryo sac, whereby a functional connection is established and, at the same time, the transportation of nutrients ensured.

Here and there the fine plasm threads bunch together and form triangular or square compact structures. On the surface, in contact with the vacuole, a bordering membrane is

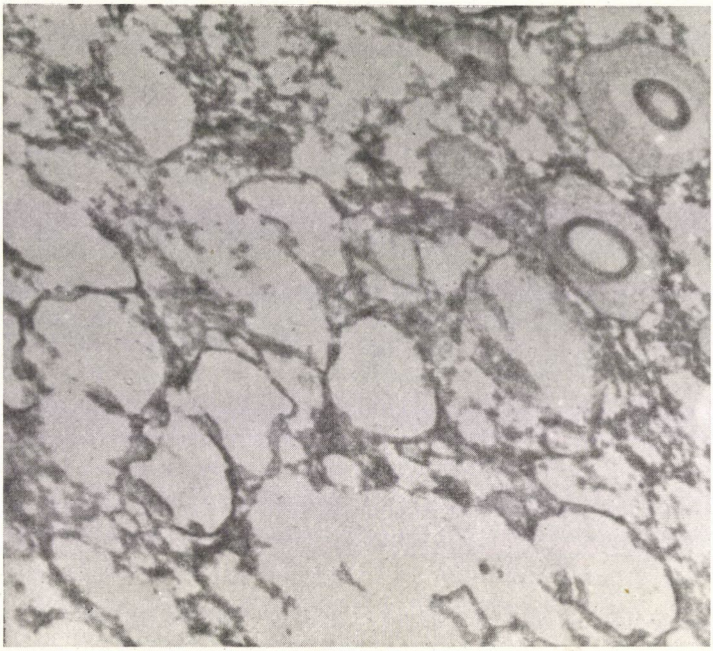

Fig. 2. Electron- microscopic photo of the vacuole of the embryo sac, with a fine plasmic network and leucoplasts (2800×)

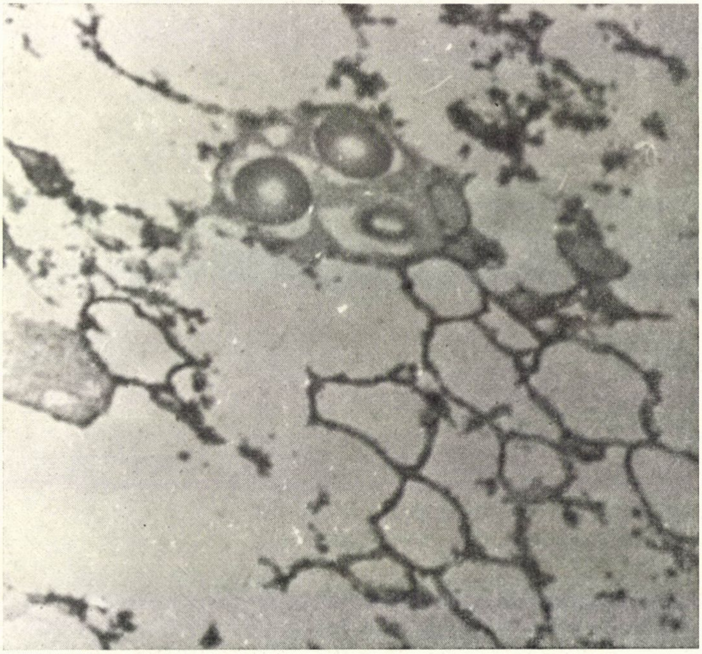

Fig. 3. When further magnified, in the cytoplasmic structure of the vacuole a larger leuoplast can be seen with three starch grains (9000×)

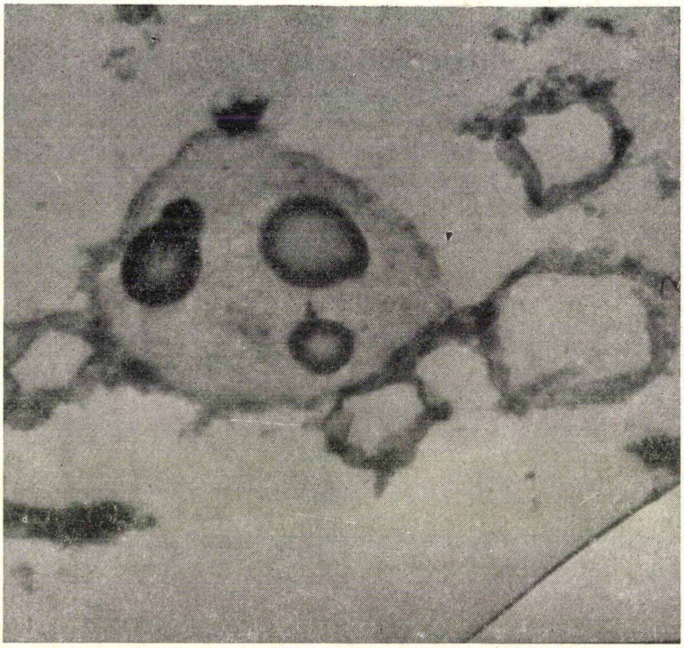

Fig. 4. The fine thready structure of the leucoplast includes three dark starch grains (21, 000×)

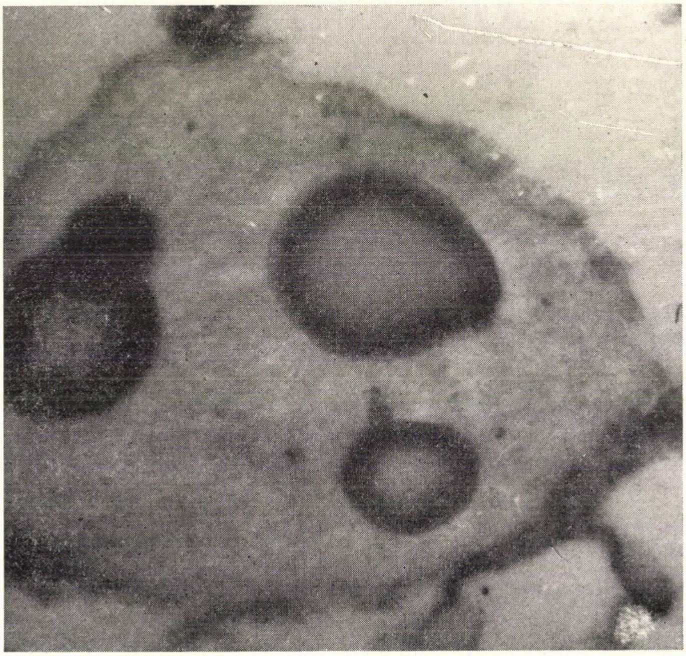

Fig. 5. Leucoplast further magnified (30, 000×)

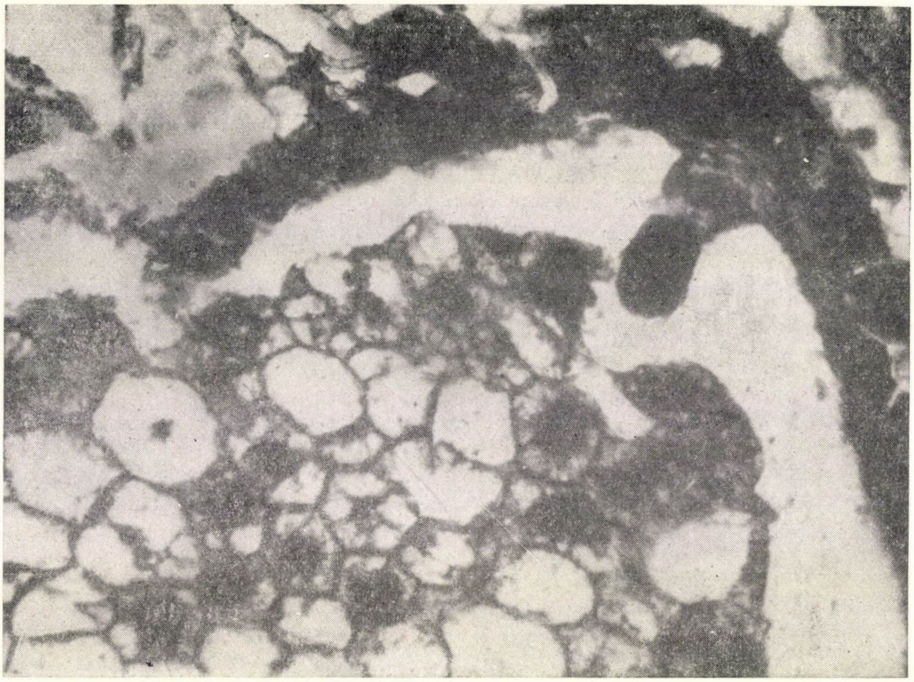

Fig. 6. Part of the endospermium (already cellular) with fully developed cell-walls (Obj. 40× oc. 4×)

found, which is somewhat thicker than the fine thready structure interweaving this inner part, and disclosing two or three egg-shaped bodies of darker colour. These show properties characteristic of starch grains, so the whole structure is regarded as a leucoplast, and since it produces starch, can be called amyloplast (Figs 3, 4 and 5). The development of starch in the embryo sac produces a readily mobilizable nutrient reserve which is used up — probably early — for the organization of the endosperm and embryo.

The fine network of cytoplasm disclosed in the embryo sac by electron- microscope examinations also promotes the further organization processes, e.g. the organization of endosperm and embryo, better than the structureless vacuolar system known so far, namely the fine cytoplasm network provides a connection with the cells surrounding the embryo sac. In the vacuole interwoven with cytoplasm various processes take place; after the fertilization the soluble central nucleus is drawn to the antipodes, then undergoes a repeated divison, and in the perimetric zone of the embryo sac, at the edge of the vacuole, a nuclear endosperm develops. The wall-less nuclei get into the vacuolar part interwoven with fine cytoplasm, and form a nuclear tapetum. In the course of the further organization the cellular endosperm will also be formed here, and the fine structure of the cytoplasm probably contributes to the development of the cell-walls.

To summarize the results of our investigations, the large vacuole of the embryo sac found by the light microscope to be almost structureless, was shown by electron- microscope observations to be interwoven with a fine network of cytoplasm with amyloplasts containing nutrient reserves.

Prepared at the Department of Apllied Botany and Histogenesis of the Eötvös Loránd University, Budapest

P. Gracza, L. Fridvalszky

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GROWTH AND NITROGEN FIXATION BY BLUE-GREEN ALGA, TOLYPOTHRIX TENUIS, AS AFFECTED BY PHOSPHORUS CONTENT OF MEDIA

The beneficial effect of nitrogen-fixing blue-green algae in paddy soils has been ascertained (ALLEN 1956, WATANABE 1954, 1962, EL-NAWAWY *et al.* 1958). However, the optimum conditions for practical application in rice fields have not yet been settled. These conditions comprise the addition of different chemical fertilizers, e.g., nitrogenous and phosphatic fertilizers, type of soil and the activity of soil microflora. Such conditions play an important role in rendering the algal constituents available to rice plants.

The present work was carried out as a pre-study of the effect of phosphorus concentration on the growth of *Tolypothrix tenuis* and its ability to fix atmospheric nitrogen in pure cultures before the application of blue-green algae to paddy soils.

Alga culture: The strain of *Tolypothrix tenuis* used was obtained from the National Institute of Agricultural Science, Nichigahara, Kitako, Tokyo.

Medium; The basic medium of WATANABE *et al.* (1951), as modified by EL-NAWAWY *et al.* (1958), was employed, but lacking the phosphate source. Each litre of the medium contained: 0.2 g $MgSO_4$, 0.2 g K_2SO_4, 0.1 g $CaCO_3$, 2 ml of 1% $FeCl_3$, 2.0 g glucose, 1.0 ml microelements solution. The pH of the medium was adjusted to 7.4.

Procedure: The experiments were carried out, in triplicate in 250 ml — conical flasks containing 50 ml— aliquots of the medium. After sterilization for 15 minutes at 15 p.s.i. sterilized aliquots of K_2HPO_4 solution were then added to the flasks under aseptic conditions. These aliquots were added to give one of the following concentrations of P_2O_5; 0, 12, 36, 60 and 120 ppm. Since the basic medium contained 3 ppm of P_2O_5 due to the presence of some phosphates in its consituents, the final concentrations of P_2O_5 were 3, 13, 39, 63 and 123 ppm, respectively. The media were inoculated with a loopful inocula of *Tolypothrix tenuis* from a slant culture (21 days age) and incubated under both direct and indirect sunlight at the laboratory at 25—35 °C. After an eight-week incubation, the algal growth was filtered, washed, dried and weighed.

The total nitrogen in the algal growth was determined by the semi-micro Kjeldahl method. Soluble nitrogen in the filtrate was determined by a method given by JACKSON (1958). The total P_2O_5 content of the alga growth was determined by the method of JOHNSON—ULRICH (1959).

Effect of P_2O_5 concentration on growth of *Tolypothrix tenuis* and its ability to fix nitrogen: Consulting Table 1, there is an obvious increase in the dry weight of the alga, with

Table 1

Effect of P_2O_5 concentration on the growth of T. tenuis and its efficiency in fixing nitrogen

P_2O_5 concentration ppm	Dry weight alga g/l medium	Total nitrogen in algal ilaments		Soluble N mg/l	Total N fixed ppm	N fixed % 144.4*
		mg/l medium	%			
3	1.2	56.4	4.7	2.7	59.1	40.9
15	2.2	136.4	6.2	6.6	143.0	99.3
39	1.9	133.0	7.0	11.4	144.4*	100.0
63	2.1	126.0	6.0	9.4	135.4	93.8
123	1.7	102.0	6.0	5.2	107.2	74.2

* 144.4 = maximum T.N. fixed in the experiment

the increase in the concentration of P_2O_5 from 3 ppm to 123 ppm. At 123 ppm P_2O_5 the algal growth was relatively lower than that at the preceding concentration of 63 ppm. The nitrogen fixed by the algae reached a maximum with 15 ppm P_2O_5 (143 ppm N) without a significant difference from that obtained with 39 ppm P_2O_5 (144 ppm N); above which a pronounced reduction in N fixation took place. The depressing effect was more obvious on the percentage of the nitrogen fixed than the total growth of the alga. The nitrogen fixed in the presence of 123 P_2O_5 amounted to 74.2% of that obtained with 39 ppm.

Effect of added P_2O_5 on the algal filaments' content of P_2O_5. The data presented in Table 2 indicated that the algae could utilize increased concentrations of P_2O_5. This was evidenced by the P_2O_5 content of those algal filaments, where the percentage was 0.098 and 0.566 in the presence of 3 and 123 ppm of P_2O_5, respectively.

Table 2

Effect of variation of P_2O_5 in the medium on dry weight and total P_2O_5 in algal filaments

Conc. of P_2O_5 in the medium (ppm)	Dry wt. of alga (g/l)	P_2O_5 in alga	
		Total (mg)	%
3	1.2	1.18	0.098
15	2.2	5.25	0.243
39	1.9	6.33	0.333
63	2.1	9.66	0.460
123	1.7	9.62	0.566

The previous series of laboratory, pot and field studies on blue-green alga, *Tolypothrix tenuis*, showed that this alga increases the yield of rice and soil nitrogen. However, an addition of calcium superphosphate apparently restricted nitrogen fixation (ABOU EL-FADL *et al* 1964, 1967).

This phenomenon had been interpreted as probably due to the toxicity of the hydrogen sulphide which may be produced through the microbial reduction of the sulphate present in calcium superphosphate (ABOU EL-FADL *et al.* 1964). Working with laboratory mixed cultures of algae, it was found that high levels of P_2O_5 (90—180 *LL*m) favoured growth of the green algae, while depressed growth of the blue-green algae (ABOU EL-FADL *et al.* 1967).

The present study on the effect of P_2O_5 on a pure culture of *Tolypothrix tenuis* showed that the growth of this organism and its ability to fix nitrogen increased considerably with increasing amounts of P_2O_5 from 15 to 63 ppm. In the presence of 123 ppm of P_2O_5, both growth and nitrogen fixation were decreased. However, there was no relation between the depressing effect of a high concentration of P_2O_5 and the phosphorus consumption by the alga. The P_2O_5 contents of the alga filaments were shown to bear a definite relationship to the available P_2O_5 in the media. TAHA (1963) found a similar effect of P_2O_5 on the growth and nitrogen fixation of the algae *Hapalosiphon fontinalis*, *Anabaena variabilis* and *Calothrix elenkinii;* a significant decrease in their growth was noticed when the P_2O_5 concentration in the media increased from 8 to 79.2 ppm.

However, as the level of P_2O_5 in UAR soil is about 25 ppm, thus the bad effect of relatively high amounts of P is not due to its bad effect on the nitrogen fixing blue-green algae, but to its higher compatability with other competing micro-orgamisms.

As the role of phosphorus in the process of nitrogen fixation by blue green-algae is still unclear, attention must be given to the following considerations:

a) The response of rice to phosphorus application is not significant in most cases, especially when it is preceded by wheat or barley (GRACIE—KHALIL 1948). On the other hand, rice preceded by legumes was found to give more yield when fertilized with nitrogen than when fertilized with both N and P. The situation is not the same when considering paddy non-legumes and thus it is recommended not to use phosphorus for paddy after legumes (EID *et al.* 1962).

b) Several trials have been made for a reliable chemical method for determining the available fraction of soil phosphorus. Such a method would be a great aid in assaying soil fertility with regard to phosphorus (EID *et al.* 1962a).

c) The penetration of phosphate in the soil is nil, since most of the Egyptian soils are known to have considerable amounts of calcium carbonate and soluble calcium. Thus the efficiency of superphosphate fertilization is expected to increase if placed as pillets closer to the root region (EID *et al.* 1962b).

It was reported that 80 per cent of the nitrogen fixation by the algae takes place during the first 4 weeks that succeed the transplantation of the rice seedlings and the inoculation with the algae (DEMANDEL 1956). Thus, it is possible to delay the time of superphosphate application up to 4 weeks after transplanting, to avoid its harmful effect on the nitrogen fixation by the algae. This recommendation is approved by the plant nutritionists, as it will give the same effect on rice yield.

Faced with the afore-mentioned findings, some field experiments seem necessary. The status of the available phosphorus of the soil, the calcium superphosphate effects in moving between soil horizons, the time and rate of superphosphate application and certain microbiological activities, etc., must be investigated.

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Prepared at the Agricultural Microbiology Research Division, Ministry of Agriculture, Cairo.

A. S. EL-NAWAWY, R. M. KAMAL, M. A. EL-FADL

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FRUIT- BODY PRODUCTION OF AGROCYBE AEGERITA (BRIG) SING ON CULTURE MEDIA OF VARIOUS NITROGEN SOURCES

The *Agrocybe aegerita* is a tasteful edible mushroom which, although less known in Hungary, occurs in the southern regions in groups mainly on poplar and willow trunks from spring to autumn. The pileus is of 3—10 cm diameter, yellowish-rusty or yellowish-brown colour with whitish, occasionally splitting edges and a more or less wrinkled surface. The lamallae are pale yellow and later become brown. The stalk is 5—15 cm in length, 6—15 mm in diameter, veiled, white, though brownish at the base, the upper part is fibrous, while the lower part is covered with scales. The flesh is white but at the base, inside the stalk, brownish and tough. The sporange is pale brownish, elliptic or kidney-shaped, smooth, 9—11 × 6—7 micron in size, with a hardly visible micropyle; the spores are of dark dirty brownish colour and a special smell (BOHUS—KALMÁR—UBRIZSY 1951, HENNIG 1967). The mushroom is grown in China in a so-called semi-culture, while in Italy mainly on poplar trunks (SINGER 1961). Since the possibilities of utilization are given in Hungary, the present paper is aimed at providing information on the characteristics of the mushroom. The examined strain of *Agrocybe aegerita* was studied under laboratory conditions on a liquid agar-culture medium of 150 ml placed in a 500 ml Erlenmeyer test-tube, with ten different (eight inorganic and two organic) nitrogen sources, according to the following aspects: 1. The time the mushroom took to overgrow the surface of the culture medium 2. The time from which, the number of periods and the time to which it developed fruit bodies during the sixty days of the experiment 3. The number of normal and abnormal fruit bodies produced in the first wave and throughout the whole period of examination 4. The degree to which the initial hydrogen-ion concentration of the culture medium changed by the end of the experiment as a result of not having been stabilized with a buffer.

We found earlier that this fungus strain had often produced fruit bodies in test-tubes containing various culture media, therefore we considered it suitable for being used as a model for studying the fruit- body production of basidial fungi. By our work we wish to contribute to the knowledge of the nitrogen turnover of the fungus strain studied.

No data have been found in the literature available on the nitrogen turnover of *Agrocybe aegerita.* On the other hand, many publications give account of the nitrogen utilization of other fungi. We refer here to papers by TRESCHOW (1944), CASIMIR—HEINEMANN (1953), BOHUS—HELTAY—WONNESCH (1954), as well as to the outstanding work of BOHUS (1960) which all deal with the nitrogen utilization of *Agaricus bisporus* (Lange) Sing. too. Dry- matter production on various sources of nitrogen was studied by LAIHO (1970) in *Paxillus involutus* (Betsch) Fr., and by HACSKAYLO—LILLY—BARNETT (1954) in fourteen basidial fungi. As for the cultivated mushrooms, man has obviously become quite familiar with their nutrient turnover.

A survey of the literature has made it clear that — since in the nitrogen turnover of basidial fungi very few correlations are known reliably — it would be useful to study this process in other groups, such as in the group of the so-called wood-destroying fungi too.

The examined fungus *Agrocybe aegerita* (Brig) Sing has numerous synonimous names, such as *A. cylindracea* (DC. 1815 ex Fr. 1831) R. Maire 1938, *A. brigantii* Fr. 1863, *Pholiota cylindracea* Fr., *P. aegerita* Brif., *P. pudica* Fr. (MICHAEL—HENNIG 1967,- BOHUS—KALMÁR—UBRIZSY 1951, SINGER 1961). According to the taxonomy recommended by UBRIZSY—VÖRÖS (1964) it is classified into the family of *Agaricaceae*, order of *Agaricales*, collective order of *Hymenomycetes*, subclass of *Homobasidiomycetes*, class of *Basidiomycetes*, sub-phylum of *Eumycotina* and phylum of *Mycota.*

The examined fungus strain was developed from the pseudo-tissues of the mycelium. The culture is maintained in our laboratory under the name Aae. strain No. 8. on a malted agar-slant-culture medium labelled 32/1 (Table 1).

The liquid agar culture media used in the experiment are presented in Table 1. On the different culture media the amount of the nitrogen source was fixed at a concentration of 425 mg N/lit. in each case. Erlenmayer test-tubes of 500 ml capacity were filled, each with 150 ml of the culture medium. The test-tubes were closed with cotton plugs according to the method used in microbiology. The culture medium was sterilized for 30 minutes at an overpressure of 1 atmosphere. The urea used for the culture medium No. 26/10 was sterilized cold, by filtering it through a Seitz EK filter.

In treatments 26/1, 26/2, 26/4, 26/5 $(NH_4)^+$ ion, in treatment 26/3 $(NH_4)^+$ and NO_3^- ions, in treatments 26/6, 26/7, 26/8 NO_3^- ion, in treatments 26/9 and 26/10 the NH_2 group represented the source of nitrogen. As regards the other nutrient sources, the culture media were identical. As usual, the prepared culture media were inoculated at the centre with a piece of mycelium-interwoven agar taken from the 10- day-old malted agar-slant culture 32/1 of the strain Aae. No. 8, with four replications each. The cultures were incubated on a laboratory table at 22 °C room temperature, in natural disperse daylight and in dark at night. The free surface of the culture media in the test-tubes was a total area of 3318 mm^2, while the surface area of the inoculum was 19.6 mm^2. Thus a circular surface of 3299 mm^2 of culture medium was available for the inoculum to grow on.

Table 1

Composition of culture media used in the experiment

Culture medium	unit.	Numbers of culture media										
		26/1	26/2	26/3	26/4	26/5	26/6	26/7	26/8	26/9	26/10	32/1*
malt extract	g	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
$(NH_4)_2HPO_4$	g	2.00	—	—	—	—	—	—	—	—	—	—
$NH_4H_2PO_4$	g	—	3.49	—	—	—	—	—	—	—	—	—
NH_4NO_3	g	—	—	1.21	—	—	—	—	—	—	—	—
NH_4Cl	g	—	—	—	1.59	—	—	—	—	—	—	—
$(NH_4)_2SO_4$	g	—	—	—	—	2.00	—	—	—	—	—	2.00
$NaNO_3$	g	—	—	—	—	—	2.58	—	—	—	—	—
KNO_3	g	—	—	—	—	—	—	3.06	—	—	—	—
$Ca(NO_3)_2$	g	—	—	—	—	—	—	—	2.49	—	—	—
Richter peptone	g	—	—	—	—	—	—	—	—	3.50	—	—
urea	g	—	—	—	—	—	—	—	—	—	0.91	—
KH_2PO_4	g	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00
K_2HPO_4	g	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
$MgSO_4 \cdot 7H_2O$	g	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	g	—	—	—	—	—	—	—	—	—	—	0.10
$CaCl_2$	g	—	—	—	—	—	—	—	—	—	—	0.10
0.1% $ZnSO_4$	ml	—	—	—	—	—	—	—	—	—	—	0.50
1% $FeSO_4$	ml	—	—	—	—	—	—	—	—	—	—	1.00
1% $MnCl_2$	ml	—	—	—	—	—	—	—	—	—	—	1.00
0.01% $CuSO_4$	ml	—	—	—	—	—	—	—	—	—	—	1.00
agar-agar	g	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	20.00
distilled water	l	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
pH		6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	6.10	5.50

* Culture medium used for the maintenance of the *Agrocybe aegerita* strain Aae. No. 8.

The cultures were checked every day. The diameter of the thalli was regularly measured up to the time they totally covered the free surface. We followed each test-tube with attention to find out whether the growth of the thallum was of an aerial mycelium or submersed type. The time the fruit bodies appeared, the pilei opened and the cultures became ripe, was registered, then the fruit bodies removed and counted under sterile conditions. Cultures in which the large proportion of the pilei were normally spread, were considered mature. The removed mushrooms were dried at 105 °C, and their dry weight determined separately for each test-tube in mg dry matter/150 ml culture medium.

Deformed mushrooms differing from the normal ones were considered abnormal and counted separately; their data are also presented separately. However, the abnormal mushrooms are included, both in the total number and weight of the mushrooms. At the end of the experiment the pH of the culture medium was electrometrically measured and registered for each cuture. The period of the experiment was 60 days during which the mushrooms were regularly picked and their data processed. From the results of measuring further quantitative properties were established by calculations.

From the data obtained by measuring and calculations, the biometrical analysis of the experiment was carried out according to Sváb (1967). From this point of view the experiment was regarded as a unifactorial model — for only the quality of the nitrogen source was different in the treatments — arranged in a random block design — as the test-tubes were incubated in a random block design at the same place, under identical conditions — generally with four, and in two treatments with three replications per treatment.

The inoculated pieces began to grow within 1—2 days. The free surface of the available culture medium was overgrown in 13 days in the case of nine nitrogen sources by aerial mycelia, on the $Ca(NO_3)_2$ N-source in a submersed way; the aerial type developed a thick cover of mycelium, the submersed type a thin aerial and a thick submersed mycelium. The average number of incubation days required for the cultures to overgrow the free surface of the culture media, as well as the types of the thalli developed, are presented in Table 2. Significant differences between the experimental results as a response to various N sources were found at the level of $P = 1\%$. The shortest period in which the cultures overgrew the surface of the culture medium was 9—9.5 days of incubation, with KNO_3, NH_2CONH_2 and $NaNO_3$ used as nitrogen sources; in the case of the other N sources the number of incubation days required was 10.5—13. As regards the value of S. D. $5\% = 0.82$, the effect of N sources on the rate at which the cultures overgrew the surface of the medium, was identical with culture media containing KNO_3, NH_2CONH_2 and Na_2NO_3, as well as on the Richter peptone culture media containing NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, NH_4NO_3. The significantly lowest rate of growth found on the culture medium $Ca(NO_3)_2$ was not observed with other culture media, but the character of the thallum as compared to that of the other treatments was submersed here. It was remarkable that the organic nitrogen always increased the rate of growth, whereas the NO_3^- nitrogen when occurring with K^+ or Na^+ increased, while with NH_4^+ and Ca^{++} decreased it. The NH_4^+ cation significantly slowed down the growth of the cultures even in the presence of NO_3^- when compared with the results obtained in the case of the other NO_3^- forms. This, however, only caused a 1—2 days' loss of time in overgrowing the free surface of the culture medium. The examined two sources of organic nitrogen did not show the same behaviour as regards the growth of the mycelium. From the point of view of production and fermentation technology, the effect of the nitrogen sources KNO_3, NH_2CONH_2 and $NaNO_3$ is considered more favourable than that of the other nitrogen sources applied. It was found, further, that, while according to Treschow (1944) and Bohus *et al.* (1954), the *Agaricus bisporus* did not utilize the NO_3^- nitrogen source when developing the mycelium, *Agrocybe aegerita* made a good use of the NO_3^- nitrogen as well. Otherwise, the strain studied by us used $(NH_4)^+$ and organic nitrogen for the growth of the mycelium in the same way as *Agaricus bisporus* did.

Table 2

Average number of incubation days required for the cultures to overgrow the free surface of the culture medium, and character of the developed thallum on the different N sources

Treatment	Result	
Culture medium number N-source	Number of incubation days $\bar{x}$	Character of the thallum
26/7 KNO_3	9.0	aerial mycelium thick hyphae
26/10 NH_2CONH_2	9.0	aerial mycelium thick hyphae
26/6 $NaNO_3$	9.5	aerial mycelium thick hyphae
26/4 NH_4Cl	10.5	aerial mycelium thick hyphae
26/5 $(NH_4)_2SO_4$	10.5	aerial mycelium thick hyphae
26/1 $(NH_4)_2HPO_4$	11.0	aerial mycelium thick hyphae
26/2 $NH_4H_2PO_4$	11.0	aerial mycelium thick hyphae
26/3 NH_4NO_3	11 0	aerial mycelium thick hyphae
26/9 Richter peptone	11.0	aerial mycelium thick hyphae
26/8 $Ca(NO_3)_2$	13.0	submersed thick hyphae
$SD_{5\%}$	0.818	

During the sixty days of the experiment fruit bodies developed in almost every culture. On the culture medium containing $Ca(NO_3)_2$ as nitrogen source the strain used in the experiment did not become fruiting in any culture. In the fruiting cultures the fruit bodies appeared periodically, more than once in 37—38 cases, except for one culture (one replication) in one of the treatments. The first ripe fruit bodies were picked between the 23rd and 44th days of incubation. The incubation time required for the first fruit bodies to appear on a given culture substrate is an important characteristic of the mushroom. Table 3 shows the days of incubation required for the first fruit bodies to attain picking maturity in the case of the different nitrogen sources. When analysing the data relative to the value S. D. 5% = 4.23—4.57, we found that the average picking time of the first yield did not vary significantly on the eight sources of nitrogen; it was between the incubation days of 24.5—26.0. With the N source NH_2CONH_2 the fruit bodies of the first fruiting period were picked on the 31st day of incuba-

Table 3

Average time (number of incubation days) of the appearance and picking of the first wave of production on the different nitrogen sources

Treatment		Result
Culture medium		Number of incubation days $\bar{x}$
number	N-source	
26/7	KNO_3	24.5
26/2	$NH_4H_2PO_4$	25.0
26/3	NH_4NO_3	25.0
26/4	NH_4Cl	25.0*
26/5	$(NH_4)_2SO_4$	25.0
26/1	$(NH_4)_2HPO_4$	25.3*
26/9	Richter peptone	25.5
26/6	$NaNO_3$	26.0
26/10	NH_2CONH_2	31.0
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	4.23** 4.57***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with full and deficient data, respectively.

tion on an average, that is, after a time of culturing significantly longer than the former ones even at the level of $P = 1\%$. In general, culture media on which the first fruiting period is shorter are more favourable from the point of view of production technology. In this respect KNO_3, $NH_4H_2PO_4$, NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, Richter peptone $NaNO_3$ were identical as nitrogen sources and more favourable then NH_2CONH_2 which caused a later development of fruit bodies. As it was mentioned before, NH_2CONH_2 was one of the most efficient nitrogen sources in promoting the cultures to overgrow the surface of the culture medium, and the ten treatments produced results significantly classifiable into three groups.

The length of time within a longer growing period — in the present case 60 days — during which fruit bodies can be picked from the different culture substrates, is an important aspect in growing mushrooms. It would be useless to judge this property irrespective of the other qualitative features. The average time of the last ripe fruit bodies appearing and being picked, as expressed in the number of incubation days, required on an average, is presented in Table 4. During the 60 days of the experiment the last fruit bodies were picked from the different cultures between the 25th and 60th day of incubation. Significant differences in this respect were found between the average results of the different N source treatments at the level of $P = 1\%$. As regards the values of S. D. $_{5\%} = 8.63$—9.32, the experiment made the following observations possible. From the culture medium containin g $NH_4H_2PO_4$ the last fruit bodies were picked on the 30th day of incubation on an average. It was from this treatment that fruit bodies were picked significantly over the shortest period. From the culture media

Table 4

Average time (number of incubation days) of the appearance and picking of the last wave of production on the different nitrogen sources

Treatment		Result
Culture medium		Number of incubation days $\bar{x}$
number	N-source	
26/2	$NH_4H_2PO_4$	30.00
26/1	$(NH_4)_2HPO_4$	42.67*
26/4	NH_4Cl	45.33*
26/6	$NaNO_3$	46.25
26/10	NH_2CONH_2	51.00
26/3	NH_4NO_3	55.25
26/7	KNO_3	56.25
26/9	Richter peptone	57.00
26/5	$(NH_4)_2SO_4$	58.25
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	8.63** 9.319***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with full and deficient data, respectively

containing $(NH_4)_2HPO_4$, NH_4Cl, $NaNO_3$ and NH_2CONH_2, respectively, fruit bodies of the last fruiting period were picked between the incubation days of 42.67 and 51 on the average of the treatments. There were no significant differences between the results obtained with the latter culture media. On the culture medium containing NH_2CONH_2 the last fruit bodies occurred on the 51st day of incubation on an average. It was only the result obtained with $NH_4H_2PO_4$ as nitrogen source that differed — being lower — significantly from the former one. With NH_2CONH_2, NH_4NO_3, KNO_3, Richter peptone, and $(NH_4)_2SO_4$ used as nitrogen sources the last fruit bodies were picked between the incubation days of 51—58.25 on the average of the treatments. On these culture media the effects of treatments could be regarded as identical. The nitrogen source NH_2CONH_2 showed the same effect as the nitrogen sources of all the treatments studied and produced fruit bodies, except for the culture medium containing $NH_4H_2PO_4$ as a nitrogen source, where the period of fructification was significantly shorter. In comparison with the nitrogen sources $(NH_4)_2HPO_4$, NH_4Cl and $NaNO_3$, the $NH_4H_2PO_4$ treatment produced fruit bodies over a significantly shorter, while the NH_4NO_3 KNO_3 Richter peptone and $(NH_4)_2SO_4$ treatments over a longer period. On the culture medium containing $(NH_4)^+$ cation the cultures generally produced fruit bodies over a relatively short period, except the $(HN_4)_2SO_4$ treatment.

During the 60 days of the experiment fructification generally occurred periodically, on more than one occasion. The number of fruiting periods observed with various nitrogen sources is an important characteristic of the different mushroom- and culture- medium combina-

Table 5

Average number of production waves in the case of different nitrogen sources used

Treatment		Result
Culture medium		Number of production waves $\bar{x}$
number	N-source	
26/2	$NH_4H_2PO_4$	1.50
26/10	NH_2CONH_2	2.25
26/4	NH_4Cl	2.33*
26/1	$(NH_4)_2HPO_4$	2.67*
26/6	$NaNO_3$	3.00
26/5	$(NH_4)_2SO_4$	3.25
26/3	NH_4NO_3	4.50
26/9	Richter peptone	4.75
26/7	KNO_3	6.25
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	1.238** 1.338***

* treatment with an insufficient number of data

* when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with full and deficient data, respectively.

tions. The average number of fruiting periods found in our experiment with the different **nitrogen** source used is shown in Table 5. Cultures producing fruit bodies were found to have 1—7 periods of fructification. Significant differences between the average results of treatments were found at the level of $P = 1\%$. As regards the value of $S. D._{5\%} = 1.24—1.34$, the results of the experiments will be discussed as follows.

From culture media containing $NH_4H_2PO_4$, NH_2CONH_2, NH_4Cl, $(NH_4)_2HPO_4$ as nitrogen sources, fruit bodies were picked in the period 1.5—2.67; no significant differences were found between the results of these treatments. Nor were there any significant differences between the results obtained with the following nitrogen sources: NH_2CONH_2, NH_4Cl, $(NH_4)_2HPO_4$, $NaNO_3$, $(NH_4)_2SO_4$ (producing fruit bodies in the period of 2.25—3.23), as well as NH_4NO_3 and Richter peptone (producing fruit bodies in the fruiting period of 4.5—4.75). On the first four, and on the second to sixth culture media, respectively, fructification occurred in a significantly lower number of periods, while in the KNO_3 treatment the number of fruiting periods was the highest. On culture media containing NH_2CONH_2 and $(NH_4)^+$, respectively, the number of producing periods was lower than on those containing NO_3^- and Richter peptone.

From the point of view of cultural practices, besides the above characteristics, the quantitive study of fruit-body production is of outstanding importance. An analysis of production in the first period of fructification is considered important, since in the practice the economic aspects occasionally only justify the exploitation of the first wave of production. The average numbers of fruit bodies picked in the first wave of production from culture media

Table 6

Number of fruit bodies produced in the first wave of production on different nitrogen sources

Treatment		Result
Culture medium		Nnmber of fruit bodies/150 ml $\bar{x}$
number	N-source	
26/10	NH_2CONH_2	15.25
26/9	Richter peptone	12.75
26/4	NH_4Cl	9.66*
26/6	$NaOH_3$	5.25
26/7	KNO_3	5.00
26/1	$(NH_4)_2HPO_4$	3.66*
26/2	$NH_4H_2PO_4$	3.50
26/3	NH_4NO_3	3.50
26/5	$(NH_4)_2SO_4$	3.25
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	6.28**
		7.87***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

containing different nitrogen sources are presented in Table 6. From the different cultures 1—36 fruit bodies per 150 ml culture medium were picked. Significant differences were found between the results of the treatments at the level of $P = 1\%$. As regards the value of $S.D._{5\%} = 6.28—7.87$, we analysed the number of fruit bodies picked from the different nitrogen sources. From culture media containing NH_2CONH_2, Richter peptone and NH_4Cl as nitrogen sources, an average number of 15.25—9.66/150 ml fruit bodies were picked: the results can be considered identical. The number of fruit bodies picked from culture media containing NH_2CONH_2 and Richter peptone was significantly higher than the number of those harvested from culture media containing $NaNO_3$, KNO_3, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, NH_4NO_3 and $(NH_4)_2SO_4$ as nitrogen sources. On the culture medium containing NH_4Cl an average number of 9.66/150 ml fruit bodies developed, not significantly differing from the results obtained with the other culture media. From culture media containing $NaNO_3$, KNO_3, $(NH_4)_2HPO_4$, NH_4NO_3 and $(NH_4)_2SO_4$ as nitrogen source, 5.25—3.25 fruit bodies per 150 ml culture medium were picked on an average. These results only differed significantly from the higher yields of the culture media containing NH_2CONH_2 and Richter peptone, respectively. The highest number of fruit bodies was obtained with the two organic nitrogen sources and with NH_4 Cl. Otherwise, treatments with $(NH_4)^+$ used as a nitrogen sources seemed to produce a lower number of fruit bodies.

Among the fruit bodies there occurred mushrooms without stem or pileus, as well as sphapeless, spherical or abnormal pseudo-tissued forms. These were counted in each culture and each treatment. The average numbers of abnormal fruit bodies occurring on the different

Table 7

Number of abnormal fruit bodies in the first wave of production on different nitrogen sources

Treatment		Result
Culture medium		Number of abnormal fruit bodies/150 ml
number	N-source	$\bar{x}$
26/9	Richter peptone	0.00
26/4	NH_4Cl	0.00*
26/7	KNO_3	0.25
26/5	$(NH_4)_2SO_4$	0.25
26/6	$NaNO_3$	0.50
26/1	$(NH_4)_2HPO_4$	0.67*
26/3	NH_4NO_3	1.75
26/2	$NH_4H_2PO_4$	2.25
26/10	NH_2CONH_2	14.00
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{10\%}$	7.06** 7.92***
	CV	450.48%

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with full and deficient data, respectively.

nitrogen sources are given in Table 7. Significant differences between the results of treatments, however, could only be demonstrated at the level of $P = 10\%$. In view of the value of $CV = 450.48\%$ we found that, as regards the number of abnormal fruit bodies, our experiment was not biometrically evaluable. We can only point out that of the average number of 15.25 fruit bodies per 150 ml picked in the first wave of production from the culture medium containing NH_2CONH_2 as a nitrogen source, 14 were of abnormal character. With Richter peptone and NH_4Cl used as nitorgen sources no abnormal fruit bodies occurred. It must be noted that the appearance of abnormal forms is probably the result of several factors acting jointly, the nitrogen source of the culture medium being only one of them; no investigations have, however, been carried out in this direction.

From the point of view of assessing the amount of yield, we consider the dry weight of the fruit bodies harvested to be the most important of all data. In our investigations on a laboratory scale the qualitative evaluation of the fruit bodies would not have been reasonable, since with the method followed in the experiment only the total production of fruit bodies or pseudo-tissued forms developing on the different N sources could be approximately assessed. The dry weights of fruit bodies picked from the different N sources in the first wave of production are included in Table 8. Significant differences between the results of the experiment were found at the level of $P = 1\%$. But considering the value of $S. D._{5\%} = 54.58—58.96$, we could establish that only considerable differences between the results could be regarded

Table 8

Dry weight of fruit bodies in the first wave of production on different nitrogen sources

Treatment		Result
Culture medium		mg/150 ml $\bar{x}$
number	N-source	
26/9	Richter peptone	243.25
26/10	NH_2CONH_2	125.00
26/2	$NH_4H_2PO_4$	123.75
26/1	$(NH_4)_2HPO_4$	101,67*
26/5	$(NH_4)_2SO_4$	100.00
26/3	NH_4NO_3	92.50
26/6	$NaNO_3$	90.00
26/7	KNO_3	78.75
26/4	NH_4Cl	73.34*
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	54.58** 58.96***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

as significant. The dry fruit-body production of the cultures ranged between 50 and 260 mg/150 ml. From the culture medium containing Richter peptone as a nitrogen source an average of 243.25 mg/150 ml dry fruit body was obtained. This outstanding result was significantly higher than any of the average results found with the nitrogen source used. No significant difference was found between the 125 and 73.34 mg/150 ml dry matter production of the other culture media.

From the number and dry weight of the fruit bodies appearing in the first wave, the average weight of the dry fruit bodies picked in the different cultures was calculated. A significant difference between the results of treatments with different nitrogen sources was only found at the level of $P = 10\%$. The results of the calculation are shown in Table 9. The value of $S. D._{10\%}$ was 20.28—21.95 mg/piece. The CV value was 70.53 percent. These data show that the results of our experiment, as regards the average dry weight of the fruit bodies, could not be evaluated biometrically. It may be mentioned, however, that the largest fruit bodies, of an average weight of 39.58 mg, were found in cultures with $NH_4H_2PO_4$ as a nitrogen source. These differed significantly from the lowest weight fruit bodies — of an average weight of 15.26—12.21 mg — picked from culture media containing NH_2CONH_2 and NH_4Cl, respectively. A large dry- weight production was found (Table 8) in the Richter peptone and NH_2CONH_2 treatments, the average weight of the fruit bodies was, however, low. A somewhat closer correlation was found between the number (Table 6) and average weight of the fruit bodies, since in the case of the Richter peptone, NH_2CONH_2, NH_4Cl and $NaNO_3$ nitrogen sources, the higher number of fruit bodies was accompained by a lower average weight, while with the nitro-

Table 9

Average dry weight of fruit bodies in the first wave of production on different nitrogen sources

Treatment		Result
Culture medium		mg/piece $\bar{x}$
number	N-source	
26/2	$NH_4H_2PO_4$	39.58
26/1	$(NH_4)_2HPO_4$	33.33*
26/5	$(NH_4)_2SO_4$	31.66
26/7	KNO_3	28.51
26/3	NH_4NO_3	28.33
26/6	$NaNO_3$	26.52
26/9	Richter peptone	24.20
26/10	NH_2CONH_2	15.26
26/4	NH_4Cl	12.21*
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{10\%}$	20.28**
		21.95***
	CV	70.53%

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

gen sources NH_4NO_3, KNO_3, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$ the lower number of fruit bodies was coupled with a higher average weight. We noticed, further, that the largest-avergage- weight fruit bodies occurred among the $(NH_4)^+$ cation treatments, although the lowest- weight fruit bodies were picked from the culture medium containing NH_4Cl. The Cl^+ anion beside the $(NH_4)^+$ cation had an unfavourable influence on the nitrogen turnover of the mushoroom.

Fruit body production per incubation day is again a quality standard in the practice. Table 10 shows the trend of the dry- fruit- body production per incubation day on different nitrogen sources in the first wave of production — i.e. in the case when only a single period of fructification is exploited. The weight of the fruit bodies collected from the individual cultures ranged between 1.8 and 10.4 mg/150 ml a day. Significant differences between the results of the treatments were found at the level of $P = 1\%$. The values of $SD_{5\%}$ were established as 2.19—2.37. On the Richter peptone source of nitrogen an average dry- fruit- body production of 9.55 mg/150 ml was measured for one day of incubation. While this maximum is significantly higher than the results obtained with the other nitrogen sources, the results of the other treatments — 4.95—2.87 mg/150 ml/incubation day — did not significantly differ from each other. The results included in Table 10 are usefully compared with the data of Table 3 where the number of incubation days required for the first period of fructification is shown. On the culture medium containing NH_2CONH_2 the yield of the first wave of production was harvested

Table 10

Average dry matter production per incubation day in the first wave of production on different nitrogen sources

Treatment		Result
Culture medium		mg/150 ml incubation day $\bar{x}$
number	N-source	
26/9	Richter peptone	9.55
26/2	$NH_4H_2PO_4$	4.95
26/10	NH_2CONH_2	4.18
26/5	$(NH_4)_2SO_4$	4.00
26/1	$(NH_4)_2HPO_4$	4.00*
26/3	NH_4NO_3	3.70
26/6	$NaNO_3$	3.46
26/7	KNO_3	3.24
26/4	NH_4Cl	2.87*
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	2.19** 2.37***

* treatment with an insufficient number of data
** when comparing treatments with a full number of data
*** when comparing treatments with deficient data, as well as with a full and deficient number of data, respectively.

on the 31st day on an average, while on the other culture media, after a significantly shorter time, 24.5—26.0 days. The quantitative values of the latter did not significantly differ from each other. On the nitrogen source NH_2CONH_2 it took an average of 31 days to produce the daily 4,95 mg/150 ml dry matter. On an operative level, in certain cases — with the single-wave production system used — this may be considered disadvantageous in comparison with the results of the other N sources. With the Richter peptone, NH_2CONH_2 and $(NH_4)^+$ cation nitrogen sources the daily production was higher than in the NO_3^- anion treatments, although the lowest yield was produced by the NH_4Cl nitrogen source; in this case, however, the presence of the Cl^- anion cannot be left out of consideration.

The average numbers of fruit bodies picked from the different nitrogen sources during the sixty days of the experiment are presented in Table 11. The producing cultures yielded 6—73 fruit bodies per 150 ml culture medium. Significant differences between the results of the treatments were found at the level of $P = 1\%$. At $P = 5\%$ the significant difference was 4,23—4,57/150 ml. When comparing the results with those obtained in the first wave, we find a higher number of fruit bodies occurring in the Richter peptone and NH_2CONH_2 treatments, and a lower number in the $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ treatments than in the other ones by the end of experiment. In comparison with the other nitrogen sources, on KNO_3 a moderate number of fruit bodies occurred in the first wave and a considerable number by the end of the experiment.

On Richter peptone an average number of 44.75/150 ml fruit bodies developed. On this culture medium the number of fruit bodies was significantly the highest compared to all

Table 11

Number of fruit bodies by the end of the experiment on different nitrogen sources

Treatment		Result
Culture medium		Number of fruit bodies/ 150 ml
number	N source	$\bar{x}$
26/9	Richter peptone	44.75
26/7	KNO_3	31.50
26/10	NH_2CONH_2	26.50
26/1	$(NH_4)_2HPO_4$	24.00*
26/3-	NH_4NO_3	23.25
26/6	$NaNO_3$	18.50
26/4	NH_4Cl	17.66*
26/5	$(NH_4)_2SO_4$	13.75
26/2	$NH_4H_2PO_4$	11.25
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	4.23** 4.57***

* treatment with an insufficient number of data
** when comparing treatments with a full number of data
*** when comparing treatments with deficient data as well as those with a full and deficient number of data, respectively.

the other culture media included in the experiment. On the KNO_3 nitrogen source the average number of fruit bodies produced was 31.5/150 ml; this result also differs significantly from the other results. In the case of NH_2CONH_2, $(NH_4)_2HPO_4$ and NH_4NO_3 26.5—23.25/150 ml fruit bodies were obtained on an average. These results can be regarded as of identical values, while on the other culture media the number of fruit bodies was significantly higher or lower. There were no evaluable differences in the results obtained either between $NaNO_3$ and NH_4Cl, or between NH_4Cl and $(NH_4)_2SO_4$, or between $(NH_4)_2SO_4$ and $NH_4H_2PO_4$, but the values decreased significantly in the above order of succession. The results further show that once again generally less fruit bodies were obtained with NH_4^+ used as nitrogen source than when NO_3^- or organic N sources were employed.

The average numbers of fruit bodies considered abnormal on the different nitrogen sources are presented in Table 12. The value of $SD_{10\%} = 7.14—7.7$ was only significant at $P = 10\%$, while the value of CV was extraordinarily high: 270 per cent. These data do not render it possible for us to make a biometric analysis of the effect of various nitrogen sources on abnormal fructification. From this point of view the experiment is not considered evaluable. Nevertheless, if we compare the results with the average numbers presented in Table 7 of abnormal forms produced in the first wave, we can see that the situation regarding the occurrence of abnormal fruit bodies did not essentially change by the end of the experiment. It was only in the case of the — otherwise low yielding — NH_4Cl nitrogen source that abnormal fruit bodies were not found in the fruiting cultures. Abnormal fruit bodies were again found in the largest numbers — though only with a 90-per-cent reliability — on NH_2CONH_2. In the other treatments 0.25—2.5/150 ml abnormal fruit bodies were counted on the average.

Table 12

Average number of abnormal fruit bodies by the end of the experiment on the different nitrogen sources

Treatment		Result
Culture medium		Number of abnorml fruit bodies/150 ml $\bar{x}$
number	N source	
26/4	NH_4Cl	0.00*
26/9	Richter peptone	0.25
26/6	$NaOH_3$	0.50
26/7	KNO_3	0.50
26/5	$(NH_4)_2SO_4$	0.75
26/1	$(NH_4)_2HPO_4$	1.00*
26/2	$NH_4H_2PO_4$	2.25
26/3	NH_4NO_3	2.50
26/10	NH_2CONH_2	14.25
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{10\%}$	7.14** 7.70***
	CV	270%

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data as well as those with a full and deficient number of data, respectively

The dry weights of fruit bodies produced on the different sources of nitrogen by the end of the experiment are summed up in Table 13. The difference between the results of the treatments was significant even at the level of $P = 1\%$. According to our calculations, the values of $SD_{5\%}$ were 102.29—104.7 mg/150 ml. The dry weight of fruit bodies picked from the producing cultures was 88—645 mg/150 ml. On culture media containg Richter peptone as nitrogen source the dry weight of the fruit bodies produced was 592.0 mg/150 ml on an average. In the other treatments the dry weights of the fruit bodies were significantly lower. On the nitrogen sources KNO_3 and NH_4NO_3 the dry weight production was 371.5—368.25 mg/150 ml on an average. These results did not significantly differ from each other, but were lower than the values obtained with Richter peptone and higher than those recorded for the other culture media. In the case of the nitrogen source NH_2CONH_2 an average dry fruit- body weight of 264.25 mg/150 ml was obtained. This can be considered identical with the production of the nitrogen sources $NaNO_3$ and $NH_4H_2PO_4$, but is significantly lower than the values obtained with NH_4NO_3, KNO_3 and Richter peptone and higher than those attained with $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and NH_4Cl. From culture media containing $NaNO_3$ and $NH_4H_2PO_4$ fruit bodies of an average dry weight of 216.5 mg/150 ml were picked. These values did not differ from the fruit- body production of NH_2CONH_2, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and NH_4Cl but were significantly lower than those attained with the nitrogen sources NH_4NO_3, KNO_3 and Richter peptone. Treatments $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and NH_4Cl produced fruit bodies of an average dry

Table 13

Total dry weight of fruit bodies by the end of the experiment on the different nitrogen sources

Treatment		Result
Culture medium		mg/150 ml $\bar{x}$
number	N source	
26/9	Richter peptone	593.00
26/7	KNO_3	371.50
26/3	NH_4NO_3	368.25
26/10	NH_2CONH_2	264.25
26/6	$NaNO_3$	216.25
26/2	$NH_4H_2PO_4$	172.50
26/5	$(NH_4)_2SO_4$	161.25
26/1	$(NH_4)_2HPO_4$	136.70*
26/4	NH_4Cl	129.30*
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	102.29** 104.70***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

weight of 161.25—129.3 mg/150 ml. The production of the nitrogen sources $NH_4H_2PO_4$ and $NaNO_3$ did not significantly differ from the latter values, but the amount of dry fruit bodies was significantly higher in the NH_2CONH_2, NH_4NO_3, KNO_3 and Richter peptone treatments.

When comparing the data of Table 8 with those presented in Table 13 we find the yield of the Richter peptone nitrogen source to have been the highest in the first wave as well. However, in comparison with the summarized results of NH_2CONH_2 it is in the group of medium large yields. By the end of the experiment the average dry weights of the fruit bodies produced on the nitrogen sources KNO_3 and NH_4NO_3 attained the level of the large yields, though in the first wave they had only taken the sixth and eighth place, respectively. The NH_4Cl treatment produced the lowest dry weight of fruit bodies both in the first and the subsequent periods of fructification. While of the inorganic N sources $(NH_4)^+$ cations gave higher yields in the first wave, by the end of the experiment the NO_3^- treatments proved to be more efficient.

The average dry weight of the fruit bodies was also calculated from weighing results summarized according to cultures in the course of the experiment. The average dry weight of the fruit bodies ranged between 2.16 and 87.5 mg. The average weights of the fruit bodies picked from different nitrogen sources are contained in Table 14. No significant differences were found between the results of the treatments even at the level of $P = 10\%$. The CV value was 97.85%. This indicates that our experiment was not suitable for demonstrating the effect of various nitrogen sources on the average dry weight of the fruit bodies. Comparing the data of Table 14 with those of Table 9, we can see that during the first production wave, a significant difference could still be found, however, at the $P = 10\%$ level between the results of the treat-

Table 14

Average dry weight of fruit bodies on the different nitrogen sources by the end of the experiment

Treatment		Result
Culture medium		mg/piece
number	N source	$\bar{x}$
26/2	$NH_4H_2PO_4$	34.83
26/3	NH_4NO_3	17.90
26/9	Richter peptone	14.97
26/7	KNO_3	12.95
26/5	$(NH_4)_2SO_4$	13.34
26/10	NH_2CONH_2	11.92
26/6	$NaNO_3$	11.75
26/1	$(NH_4)_2HPO_4$	10.84*
26/4	NH_4Cl	8.51*
26/8	$Ca(NO_3)_2$	did not turn into bearing

$F = 1.72$ ($F_{10\%} = 1.9$) The differences are not significant.
$CV = 97.85\%$

* treatment with an insufficient number of data

ments. The average dry weight of the fruit bodies showed the highest values using $NH_4H_2PO_4$, and the lowest using NH_4Cl as nitrogen source, taking fruit bodies picked both in the first and every period of fructification in consideration. The interrelation between the dry weights of the fruit bodies picked on the two occasions was various in the case of the other nitrogen sources. In the $NH_4H_2PO_4$, NH_2CONH_2 and NH_4Cl treatments the average fruit body dry weights found in the first wave decreased to about 88, 78 and 70 per cent; in the NH_4NO_3 and Richter peptone treatments to 63 and 62 per cent; in the KNO_3, $NaNO_3$ $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ treatments to 45, 44, 42 and 32 per cent, respectively. By the end of the growth season the average dry weight of the fruit bodies decreased considerably in all treatments. Since, as regards the average dry weights indicative of the size of the fruit bodies, the results of the experiment are not suitable for the purpose of biometric analyses, a detailed analysis of the correlations on the basis of these data did not seem reasonable.

From the fruit body dry weights totalled up to the time when the yield of the last wave of production was harvested, as well as from the number of incubation days we calculated the volume of fruit- body- dry- weight production per incubation day. As we have already mentioned, this quantitative property is a very important criterion of productivity in the practice. In production cultures the daily dry- matter production was 2.5—10.98 mg/150 ml. Table 15 shows the dry- matter production of the fruit bodies per incubation day, with various nitrogen sources used. Significant differences between the results of the treatments were found at $P = 1\%$. The value of $SD_{5\%}$ was determined at 1.86—2.01 mg/150ml /incubation day. The daily average production of 10.39 mg/150 ml found on Richter peptone was significantly the highest value throughout the whole experiment. The results obtained with KNO_3, NH_4NO_3, $NH_4H_2PO_4$ and NH_2CONH_2 — 6.63—5.01 mg/150 ml/incubation day — did not differ essentially from one another, but the daily production found on the other nitrogen sources was sig-

Table 15

Average dry-matter production per incubation day on the different nitrogen sources

Treatment		Result
Culture medium		mg/150 ml incubation day $\bar{x}$
number	N-source	
26/9	Richter peptone	10.39
26/7	KNO_3	6.63
26/3	NH_4NO_3	6.49
26/2	$NH_4H_2PO_4$	6.04
26/10	NH_2CONH_2	5.01
26/6	$NaNO_3$	4.66
26/1	$(NH_4)_2HPO_4$	3.21*
26/4	NH_2Cl	2.77*
26/5	$(NH_4)_2SO_4$	2.76
26/8	$Ca(NO_3)_2$	did not turn into bearing
	$SD_{5\%}$	1.86** 2.01***

* treatment with an insufficient number of data

** when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

nificantly higher or lower than that. The production of the nitrogen sources NH_4NO_3, KNO_3, $NH_4H_2PO_4$, NH_2CONH_2 and $NaNO_3$ — 6.49—4.66 mg/160 ml/incubation day — can similarly be considered as of identical level, on the other hand, it significantly differs from the production of the other treatments. The production of the NH_2CONH_2, apart from the previous nitrogen sources, does not differ evaluably from the daily yield of the $(NH_4)_2HPO_4$ treatment either, whereas it is significantly less than the yield of the Richter peptone and more than that of the NH_4Cl. It was only from the yield of $(NH_4)_2SO_4$, KNO_3 and Richter peptone that the daily production found with the nitrogen source $NaNO_3$ did not significantly differ. The production of the nitrogen source $(NH_4)_2HPO_4$ was significantly higher than that of $NH_4H_2PO_4$ NH_4NO_3, KNO_3 and Richter peptone and was at the same level with all the other treatments. The dry-matter production per incubation day of the nitrogen sources NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, $NaNO_3$, further of $(NH_4)_2SO_4$, NH_4Cl and $(NH_4)_2HPO_4$ was of identical level but significantly lower than the yields of the other treatments.

When comparing the results of Table 15 with the data of Table 10, we first see that Richter peptone resulted in the highest daily production throughout the whole experiment. As for the other treatments, the order of succession of treatments was generally not identical in the first wave and at the end of the experiment, only the production of the nitrogen source NH_4Cl was at a low level on both occasions. Secondly, it is conspicuous that in the case of the $(NH_4)_2HPO_4$, NH_4Cl and $(NH_4)_2SO_4$ treatments the daily dry matter production was higher in the first wave than at the end of the experiment. Thus, in the latter treatments the productivity of the cultures decreased. A similarly interesting correlation is found when the

Table 16

pH value and H^+ concentration of the culture media at the end of the incubation period

Treatment		Result	
Culture medium			
number	N source	pH	(H^+)
26/5	$(NH_4)_2SO_4$	3.03	$0.934 \cdot 10^{-3}$
26/4	NH_4Cl	3.26	$0.551 \cdot 10^{-3}$*
26/2	$NH_4H_2PO_4$	3.44	$0.367 \cdot 10^{-3}$
26/1	$(NH_4)_2HPO_4$	3.72	$0.194 \cdot 10^{-3}$*
26/9	Richter peptone	4.71	$0.197 \cdot 10^{-4}$
26/3	NH_4NO_3	5.82	$0.151 \cdot 10^{-5}$
26/8	$Ca(NO_3)_2$	6.24	$0.581 \cdot 10^{-6}$
26/10	NH_2CONH_2	6.44	$0.363 \cdot 10^{-6}$
26/6	$NaNO_3$	7.18	$0.660 \cdot 10^{-7}$
26/7	KNO_3	7.19	$0.643 \cdot 10^{-7}$
	$SD_{5\%}$		$0.246 \cdot 10^{-3}$** $0.268 \cdot 10^{-3}$***

* treatment with an insufficient number of data

* when comparing treatments with a full number of data

*** when comparing treatments with deficient data, as well as those with a full and deficient number of data, respectively.

data of Table 15 are compared with those of Table 5: a higher daily dry- matter production is usually brought about in more than one wave. In the practice optimum tests decide whether under the given conditions a surplus input due to the utilization of more than one wave of production will be compensated by a higher yield or not. It could be established that, while in the first wave generally the $(NH_4)^+$ cation, on the whole of the growth season the NO_3^- anion nitrogen sources gave the higher production results.

The pH value of the culture media used in the experiment was adjusted to 6.1 and any possible changes occurring in it during the sixty days of incubation were measured. The results are presented in Table 16. Significant differences between the results of the experiment were found at the level of $P = 1\%$. The value of $SD_{5\%}$ was determined in $0.246 \cdot 10^{-3}$—$0.268 \cdot 10^{-3}$ hydrogen- ion concentration. During the 60 days of incubation the initial pH value of 6.1 decreased to 5.95—2.9 or increased to 6.2—7.45— i.e., the cultures became more acidic or more alkaline. In the case of inorganic nitrogen sources this shift of the pH value depended on whether it was the cation or the anion of the N source that the mushroom utilized. In the former case acidification, in the latter case alkalescence occurred. These results were more or less foreseen. However, it was only the pH values of 3.44—3.03 found in the treatments $NH_4H_2PO_4$, NH_4Cl and $(NH_4)_2SO_4$ that showed a significant decrease in comparison with the initial pH value of 6.1. Between the results of the treatments the following correlations could be established. The significantly highest (H^+) concentration was attained by the culture medium containing $(NH_4)_2SO_4$. It was only from the pH value of the $NH_4H_2PO_4$ treatment that the pH value of the treatment NH_4Cl did not significantly differ. It was only from the

(H+) concentration of the treatments NH_4Cl and $(NH_4)_2HPO_4$ that the pH value measured on the nitrogen source $NH_4H_2PO_4$ did not differ. The result obtained with the nitrogen source $(NH_4)_2HPO_4$ was different from those of the significantly lower pH-value treatments containing NH_4Cl and $(NH_4)_2SO_4$. The pH values of culture media containing Richter peptone NH_4NO_3, $Ca(NO_3)_2$, NH_2CONH_2, $NaNO_3$ and KNO_3, respectively, only differed significantly from the pH values of culture media containing $NH_4H_2PO_4$, NH_4Cl and $(NH_4)_2SO_4$ · In three treatments [$(NH_4)_2SO_4$, NH_4Cl, $NH_4H_2PO_4$) with pH values fallen to the lowest level, both the number (Table 11) and the dry weight (Table 13). of the fruit bodies were relatively low.

In the two highest (H+) concentration treatments the lowest values of daily dry-matter production were also found on the culture media containing $(NH_4)_2SO_4$ and NH_4Cl as nitrogen sources (Table 15). While all the three lowest pH treatments were included in the former correlation, in the latter case the lowest production only appeared in the two most acidic treatments.

Of the inorganic nitrogen sources those containing $(NH_4)^+$ cation showed the most intensive acidification. In cultures containing NO_3^- anion and organic nitrogen, respectively, the pH value changed to a smaller degree. By the end of experiment the larger yields occurred with organic nitrogen or NO_3^- present. A comparison of the results suggested that the intensive acidification of the culture medium had an unfavourable effect on fructification. It is probable that when inorganic nitrogen sources are present, it is the acidification of the culture medium rather than the $(NH_4)^+$ and NO_3^- forms of nitrogen that has a more direct action on fructification. In the first period of production the culture media containing $(NH_4)^+$ cation has probably not become too acidic yet; in that period the yield of the $(NH_4)^+$ cation treatments was still more favourable than the production of cultures containing NO_3^- anion. A full explanation of the phenomenon requires further investigations.

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*

Prepared at the Department of Site Knowledge of the University of Forestry and Timber Industry, Sopron

T. Takács

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A MICROSCOPE FLUOROMETER WITH SHORT-TIME EXCITATION AND ELECTRONIC SHUTTER CONTROL

Owing to its high analytical sensitivity and great practicability, quantitative fluorescence microscopy has played an increasingly important role in biology and medicine in recent years. An important field of application of this method is cytofluorometry, i. e., the quantitative determination of nucleic acids, proteins, amino acids, biogenic amines, as well as substances added to cells and cell organelles for test purposes. Because of the inhomogeneity of the objects, the absorption methods applied require scanning techniques which demand a major outlay in apparatus and time and often are not suitable for the analysis of very small quantities of substance.

In cytofluorometry the quantity of a substance is determined simply by measuring the light emitted by all parts of an object in the exit pupil of a microscope. A prerequisite for this is a proportionality between substance content and fluorescence intensity in each object point, which can be achieved with suitable fluorochromes and object thicknesses. The object to be measured is defined in the image plane of the microscope. The diaphragm required generally may be larger than the object image as the surrounding field in the fluorescence microscope is dark. The analytical sensitivity depends on the type of the fluorescing substance and can strongly be increased by using intensive light sources, as well as suitable optics and light detectors. In contrast to absorption photometry, fluorometry at first supplies only relative values for substances. Absolute values are obtained by comparison measurements with objects of known substance quantity.

The basic design of a cytofluorometer is illustrated in Fig. 1. Fluorescence excitation is achieved with an intensive light source, usually a super-pressure mercury lamp or a high-pressure Xenon arc lamp, in conjunction with a brigh-field vertical illuminator. The latter contains exchangeable reflectors with chromatic beam-splitters for various spectral regions (UV, violet, blue or green excitation). Thus, optimum excitation and simultaneous dark field are obtained. Since the microscope objective also serves as condensor, in focusing the objective on the object optimum illumination is adjusted. This fact has a favourable effect on the measuring accuracy and allows fast work. Combination of the reflected-light equipment with a transmitted-light illuminator is simple. Such an illuminator is needed for scanning the specimen and centering the object in the aperture in order to avoid premature fading of the objects by the exciter light. Generally phase contrast (Ph) is best suited for this purpose. Exciter and barrier filters used are the same as in usual fluorescence microscopy. A telescope or TV equipment serves for viewing the image and aperture. By means of a field lens the exit pupil of the microscope objective is imaged in the photomultiplier.

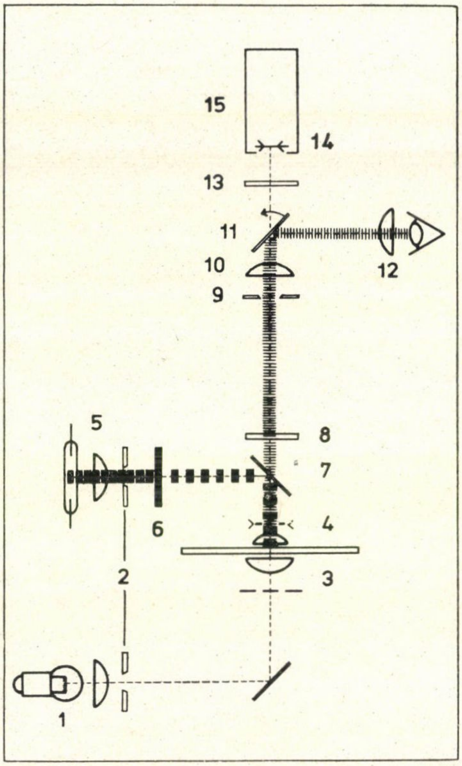


Fig. 1. Illustration of the beam path in the cytofluorometer: (1. filament lamp, 2. lamp field stops, 3. Ph condenser, 4. Ph objective, 5. mercury or Xenon lamp, 6. exciter filter, 7. reflector Fl, 8. barrier filter, 9. image plane with measuring aperture, 10. field lens, 11. folding mirror, 12. telescope, 13. interference filter monochromator, 14. pupil plane, 15. photomultiplier)

The main difficulty of cytofluorometric measurements is in the fading of the specimen during fluorescence excitation (photodecomposition). Taking dansyl chloride as an example, Fig. 2 (curve A) shows how strongly the fluorescence intensity can decrease with the excitation. This effect to a greater or lesser degree exists with all fluorochromes and makes work by the conventional method of absorption photometry extremely difficult. Even if the object is focused in transmitted light with subdued filament lamp illumination, in most cases a remarkable decrease of intensity during a measuring process lasting seconds (with galvanometers or recorders) occurs. Only when excitation and measuring time can be reduced to a small fraction of a second is fading no longer of any significance.

A further disadvantage of existing microscope photometers usually is the awkward and time-consuming operation of various control elements for each individual measurement.

In developing the OPTON Cytofluorometer close attention was paid to these factors. The recently introduced instrument (Fig. 3) operates with an excitation and measuring time of about 7 milliseconds. All controls necessary for the measuring process have been automated. Used as a base is the large fluorescence microscope with reflected-light excitation (Stand UNIVERSAL or PHOTOMICROSCOPE) and the modular electronic system for quantitative microscopy.

The microscope can be equipped with various exciter light sources. The HBO 100 mercury lamp and the XBO 150 Xenon lamp are preferred. For both types highly stabilzed power supply units are available which provide the stability of the light source required for the single-beam operation. Various exciter and barrier filters, as well as reflectors can be rapidly changed in the vertical illuminator, either singly or jointly. The vertical illuminator further contains various lamp-field stops in a centerable turret. The NEOFLUAR phase contrast objectives are best suited. There are adapters to accept fluorescence standards (uranium glass plates) in a magnetic holder for simple and rapid calibrating of measured values.

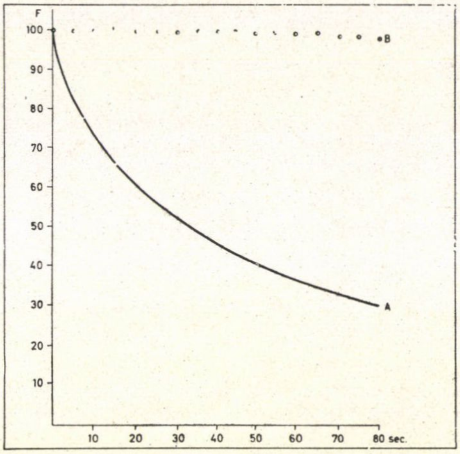


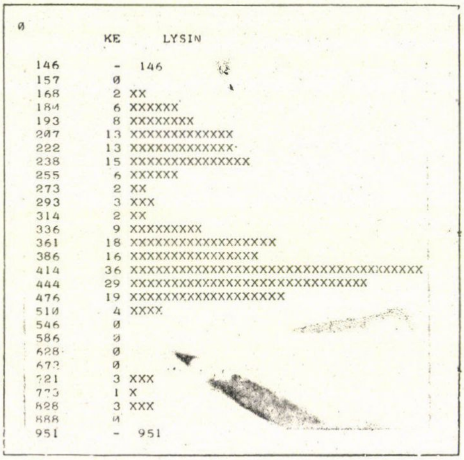
Fig. 2. Influence of fading on fluorescence measurement: (A. with continuous blue excitation-B. with blue excitation of 7 milliseconds, period at intervals of 5 seconds. Mastocytoma cells stained with dansyl chloride. Abscissa: excitation time in seconds. Ordinate: relative fluorescence intensity F.)

Special consideration was given to the requirements of cytofluorometry in developing the new microscope photometer 01. This can be directly attached on to the microscope. However, for work at high magnifications it is recommended to use the AC stand to ensure vibration-free support of the instrument. The photometer attachment 01 contains exchangeable diaphragms, a field lens (from quartz glass) for pupil imaging, a movable mirror and the photomultipler. Depending on the position of the mirror, all light is directed upwards into the multiplier, to the front into a telescope, or to the rear into a photographic or TV camera. Between the positions "observation" and "measurement" the mirror is moved by means of a rotary magnet (in a simple design of the microscope photometer 01 via a cable release). The following diaphragm inserts are provided: iris diaphragm and circular stops in revolving turret, variable rectangular diaphragms, electrically controlled circular stop insert. For fluorescence spectral measurements a continuous interference filter monochromator can be attached below the multiplier. This makes the microscope photometer 01 a universal instrument, not only for use in fluorescence measurements but also for most of the other methods of microphotometry. Since it does not feature beam splitting nor reflection in the photometric part, it is of special advantage in the examination of weakly fluorescing objects and polarized light phenomena.

An electrically controlled shutter serves to limit fluorescence excitation to about 7 milliseconds. As shown in Fig. 2 (point B), the harmful fading of the specimen is thus practically eliminated. Only after more than ten measurements of the same object does gradual diminishing of the measured value occur. During measurement the tungsten auxiliary light is interrupted by a second, electrically controlled shutter. For photometric measurement the following parts from the OPTON electronic system are required: the amplifier with stabilized high-voltage unit for the photomultiplier and decimal display unit.

For the automatic control of mirror, shutters, diaphragms, and display unit, a special electronic slide-in unit has been developed. The measuring process is as follows:

The phase contrast image of the object to be measured is centered on a cross-hair. For this purpose the focusing eyepiece of the microscope photometer or for lengthy work more conveniently a TV equipment, is used. Then the diaphragm corresponding to the object size is swung in (automatically through foot contact or manually). The object can now be checked for its location within the measuring field and re-centered, if necessary. Through the foot contact the following control sequence is triggered: tungsten auxiliary lamp out, mirror releases photomultiplier, fluorescence excitation and photometric measurement (7 milliseconds), mirror back for image viewing, pilot lamp on. Since the entire control sequence requires less than a second, the amount of time needed for each measurement merely depends on locating the specimen and centering the selected object.



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0
              KE      LYSIN

    146        -   146
    157        0
    168        2 XX
    184        6 XXXXXX
    193        8 XXXXXXXX
    207       13 XXXXXXXXXXXXX
    222       13 XXXXXXXXXXXXX
    238       15 XXXXXXXXXXXXXXX
    255        6 XXXXXX
    273        2 XX
    293        3 XXX
    314        2 XX
    336        9 XXXXXXXXX
    361       18 XXXXXXXXXXXXXXXXXX
    386       16 XXXXXXXXXXXXXXXX
    414       36 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
    444       29 XXXXXXXXXXXXXXXXXXXXXXXXXXXXX
    476       19 XXXXXXXXXXXXXXXXXXX
    510        4 XXXX
    546        0
    586        0
    628        0
    672        0
    721        3 XXX
    773        1 X
    828        3 XXX
    888        0
    951        -   951
```

Fig. 3. Histogram printed out with PDP 8 E computer. Quantities of lysin in isolated cell nuclei of the root of *Vicia faba*. [Fluorochrome: dansyl chloride. 1st column: classes of the relative fluorescence intensity (logarithmic scale). 2nd column: number of nuclei found in each class. Next to it corresponding number of X marks.]

The electronic shutter mechanism also simplifies calibration of the fluorescence values using the above described uranium glass plate. In position 100 of the program switch, the high voltage for the multiplier is changed until the decimal display unit indicates the value 100. At the slide-in unit of the electronic shutter mechanism all controls can also be actuated manually or a program selected for the control of transmittance measurements.

The OPTON cytofluorometer can be further expanded at any time and adapted to individual tasks. For instance, a printer of measured values or a desk computer can be connected to the decimal display unit. For detailed analyses of a considerable quantity of test material use of a computer in on-line operation is an advantage. With such an arrangement, e. g. histograms (Fig. 3), mean values and other statistic data can be determined with a minimum amount of time. Connection are provided for the PDP 8 and PDP 12 from Digital Equipment Corporation. In addition, accessories for object scanning of fluorescing specimens are available.

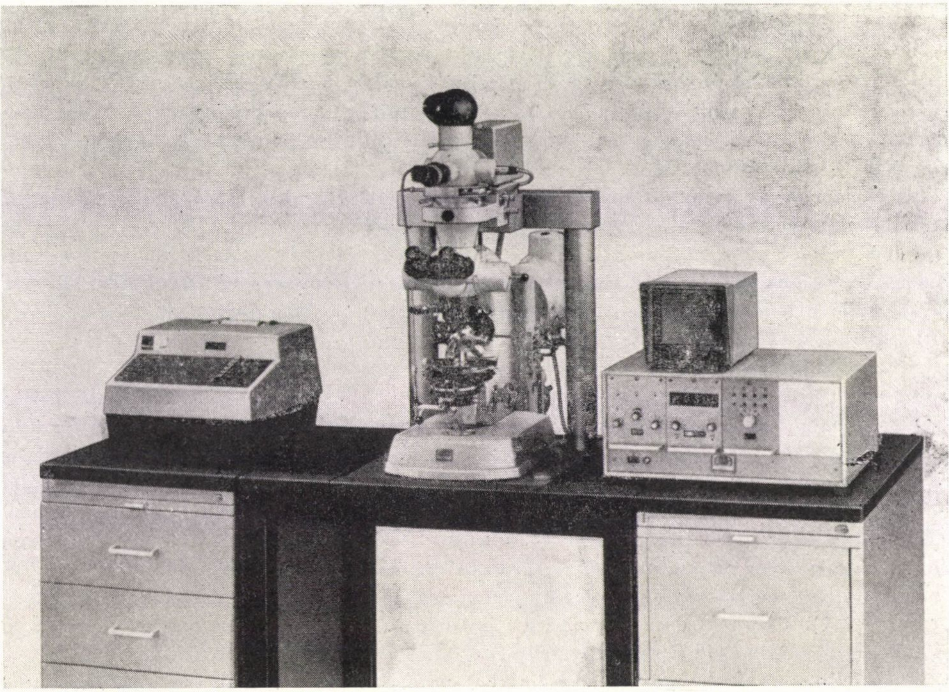

Fig. 4. OPTON Cytofluorometer. UNIVERSAL microscope with photometer attachment 01 on stand AC for fluorometric measurements with reflected-light excitation. Equipped for TV transmission of the microscopic image. Amplifier, digital display unit, electronic shutter mechanism. Kienzle printer

Althoug in developing the instrument described special attention was paid to the requirements of cytofluorometry, other fields of application have been considered. The user has the great advantage that the instrument can be used as it is, or after adding a few modules, for absorption measurements, reflectance measurements and allied methods, particularly those employing polarized light.

*

Prepared at the OPTON Feintechnik GmbH, 7082 Oberkochen (Württ).

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OCCURRENCE OF RUTIN IN SOME VIOLA SPECIES

Among the representatives of the family *Violaceae* it is *Viola odorata* L. and *Viola tricolor* L. that can primarily be taken into account from a therapeutical point of view. These plants belong to the *Nominium* and *Melanium* sections of the genus *Viola*, especially widespread in Central Europe (HEGI 1954, SOÓ—JÁVORKA 1951).

Viola odorata L. has mainly become known in therapeutics through its emetic and expectorant effect, while *Viola tricolor* L. is used in various skin diseases (eczema), further as a diuretic and mild expectorant (WEISS 1960, BRAUN 1968, BERGER 1954). *Viola arvensis* Murr. is mentioned by some authors as a variety of *Viola tricolor* L. (HEGI 1964, BERGER 1954), while others consider it an independent species. Its therapeutic application agrees with that of *Viola tricolor* L.

The *Viola* genus is generally characterized by a salicyl acid—glycoside component called violutoside; the emetic effect of some species belonging here is attributed by certain authors to salicyl acid—methyl ester, a fission product of glycoside (HEGI 1954). As for other components, the literary references are not uniform. The *Nominium* section typically represented by *Viola odorata* L. is considered to be characterized by a saponine, volatile oil and alkaloid content (HEGI 1954). Alkaloid in the root of *Viola odorata* L. was first pointed out by Bouelly (cit. BERGER 1954): according to Kroeber (in LINDE 1919) the compound is similar to the emetine of *Ipecacuanhae radix* and is supposed to be responsible for the expectorant effect of the drug, in which some role is also played by saponines unanimously demonstrated by KOFLER—STEIDL (1934). Recently, data have been published in the Polish literature on the isolation of a hypotensive alkaloid (FRENZLOWA 1961).

The literary references concerning the pigments, as, e. g. the therapeutically most frequently used violaquercitrin (rutin), are not unambiguous. According to Mandelin (cit. HEGI 1954) this compound is the most important component of *Viola tricolor* L. and *Viola arvensis* Murr. In addition, the violanine anthocyan-compound is also characteristic, which is the ramnoglycoside of delphinidine. The carotinoid violaxanthen is mainly characteristic of flowers in the examined species (KÜHN—WINTERSTEIN 1931). Literary references to saponine (ROBERG 1937), as well as alkaloid in traces — probably identical with the compound already demonstrated in *Viola odorata* L. — as contained by the representatives of the *Melanium*-section, are found only here and there (Table 1).

Our experiments were aimed at making a comparative study of the components in the mentioned three species of the genus *Viola*. Of the therapeutically important colour substances the present paper gives an account of the occurrence of violaquercitrin or rutin.

In the examinations the root and above-ground shoot of *Viola odorata* L., as well as the herb of *Viola tricolor* L. and *V. arvensis* Murr. were used as the initial material of therapeutical valuable galenics. The herb drugs partially collected or produced by us, partially purchased, mostly contained the shoots of fully developed plants.

From the drug samples we prepared a methanolic extract in a Soxhlet apparatus, and examined it tentatively for flavonoids by paper- and thin-layer chromatography (RÖMISCH 1960).

As a result of our experiments 1 flavonoid spot was found in the radix of *Viola odorata*, 2 in its herb and 3 in *Viola tricolor* and *arvensis*. The quantities of the demonstrated compounds were determined in the methanolic drug extract by the method of RÖMISCH (1960). The quantity of flavonoids was the highest in the herb of *Viola tricolor* and *arvensis* (2.1 and 1.3 per cent, respectively), much lower in the herb of *Viola odorata* (below 0.5 per cent), while in its root it was only found in traces (0.016 per cent).

Subsequently we attempted to identify the components appearing in considerable amounts on the tentative chromatograms of high flavonoid content drugs. A spot suggesting

Table 1

Components in the two sections of the genus Viola according to the literature

Nominium section		*Melanium* section	
Viola odorata L.		*Viola arvensis* Murr.	*Viola tricolor* L.
rhizoma et radix	herba	herba	herba
gaulterin	gaulterin	violaquercitrin	violaquercitrin
saponine	saponine	gaulterin	violanin
alkaloid	volatile oil	violaxanthin	violaxanthin
volatile oil	violanin?	saponine	gaulterin
iridoids?	violaquercitrin?	tanning agent	saponin
		mucilage	tanning agent
		alkaloid?	mucilage
			volatile oil
			alkaloid?

rutin was observed in the herb of both *Viola tricolor* L. and *Viola arvensis* Murr. on the chromatograms in UV light without treatment, as well as on the basis of the R_f value after development with the appropriate reagents. Considering the therapeutic importance of the compound, the subsequent experiments were aimed at isolating it and clarifying its structure. The highest-total flavonoid content *Viola tricolor* was used as model drug.

We started the work of isolation with 1 kg drug obtained by the vortical method of extraction with 15 litre ethanol. The purification of the concentrated extract made free of chlorophyll was carried out by 30 hours of continuous ethyl-acetate extraction. From the aqueous part — after standing in a refrigerator for a day — a yellow crystalline compound was separated. Having been filtered and washed in water, the crystals were recrystallized from methanol. On the basis of gravimetric measuring the obtained amount was 8.1208 g. The melting point of crystals ranged from 185 to 190 °C. This value corresponded to the melting point of rutin (Mazor 1966). To identfy the isolated compound the following examinations were performed:

By acidic hydrolysis, which resulted in the separation of the sugar fraction from the glycoside, the compound was examined partly for aglycon, and partly — after neutralization — the sugar analysis was carried out. As a result quercetin (Fig. 1), rhamnose and glucose were obtained. (Fig. 2).

Then we supported our statement concerning the aglycon fraction of the component by alkali decomposition, namely the alkali-treatment produced floroglucine and protocatechuic acid which corresponded to the fission products of quercetin. Under analkaline influence the protocatechuic acid similarly decomposed (Fig. 3).

Our experiments demonstrating aglycon were completed by taking the UV spectrum of the acidic hydrolytic product (aglycon), as well as examining the spectral shifts occurring as a response to various reagents, such as $AlCl_3$, hydrochloric $AlCl_3$, methanolic Na-acetate and methanolic Na-acetate boric acid. The results unequivocally suggested quercetin (Fig. 4), (Mabry—Markham—Thomas 1970). Hence the structure of the compound produced was unambiguously proved.

Our subsequent experiments were aimed at following the changes occurring in the rutin content during the vegetation period. In the two species mentioned the occurrence of

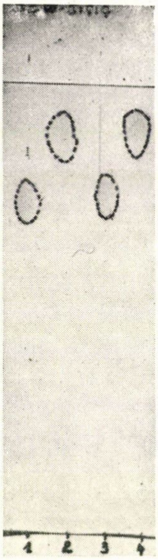

Fig. 1. Checking of acidic hydrolysate by layer chromatography. (1 = quercetin test material; 2, 4 = original glycoside; 3 = decomposition product of acidic hydrolysis; BEW = 5 : 1 : 4)

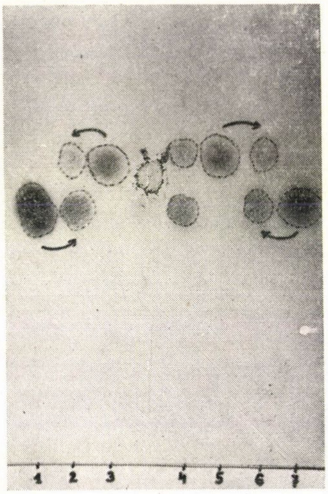

Fig. 2. Sugar analysis by paper chromatography. (1, 7 = glucose; 2, 4, 6 = material to be examined; 3, 5 = rhamnose; Butanol : Pyridin : Water = 6 : 4 : 3)

the compound was studied on a total of ten occasions, in various vegetation periods from the appearance of the first flower. Quantitative measuring was again performed according to **Römisch** (1960), by a photometric method combined with paper chromatography, as modified by us (**Pethes** 1965).

According to our experiments the rutin content — both in *Viola tricolor* L. and *Viola arvensis* L. — is the lowest when the first flower appears, (0.15 per cent), then gradually increases

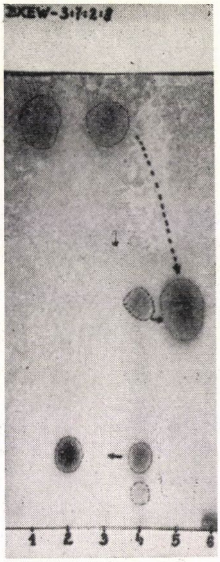

Fig. 3. Paper chromatography of alkali decomposition products. (2 = floroglucine test material; 3 = protocatechuic acid test material; 4 = end product from alkali decomposition; 5 = protocatechuic acid after alkali treatment; B : X : E : W = 3 : 7 : 2 : 8)

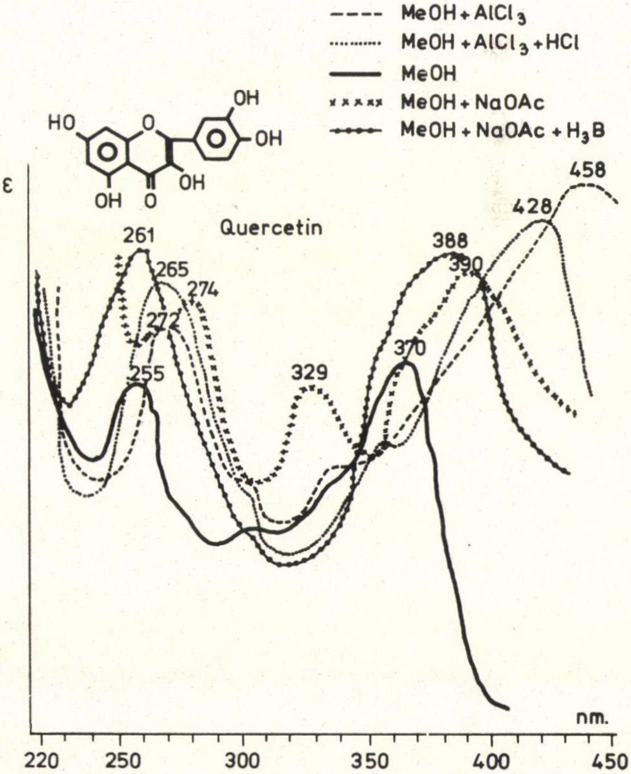

Fig. 4. UV spectrum of quercetin in various media

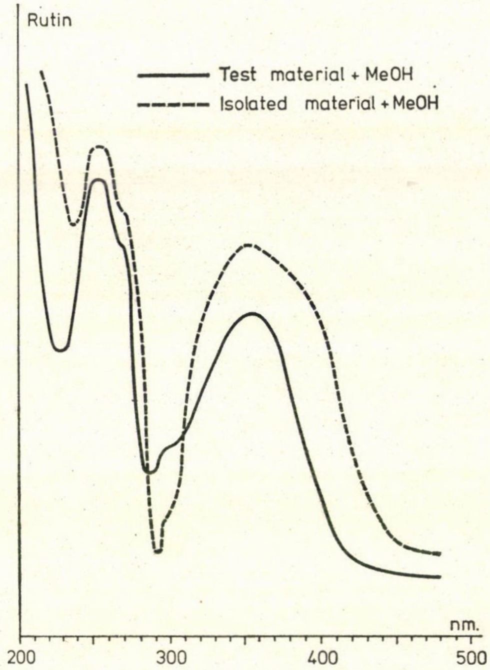

Fig. 5. UV spectrum of test rutin and rutin isolated by us in methanol

and reaches a maximum (0.45 per cent) at the multifloral, green-fruited stage followed by gradual decrease.

To sum up our investigation results, we isolated rutin from the herb of *Viola tricolor* belonging to the *Melanium* section of the genus *Viola*, and determined its structure by classical decomposition and instrumental analytical methods.

When studying the changes occurring during the vegetation period, we found the highest rutin content in green-fruited plants of *Viola arvensis* L. and *Viola tricolor* L.

Our findings confirm the experimental results of WILLSTATTER—MALLISON (cit. KOFLER GERTIG *et al.* (1966) who mention *Viola tricolor* L. and especially its flowers as definite sources of rutin. At the same time, we found *Viola odorata* L. to be poorer in flavonoid compounds.

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Prepared at the Institute of Pharmacognosy of the Semmelweis Medical University· Budapest

M. VINCZE-VERMES, G. MARCZAL, E. PETHES

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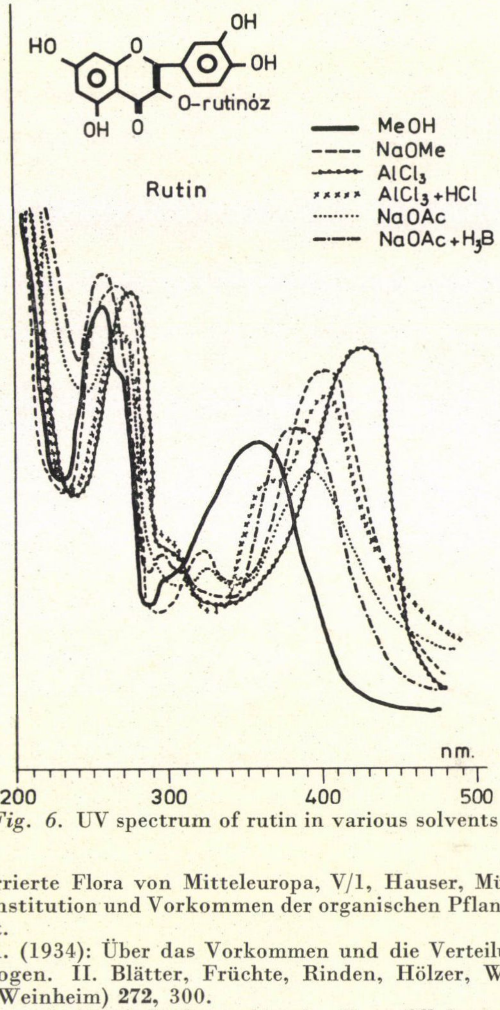

Fig. 6. UV spectrum of rutin in various solvents

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A REVIEW OF THE WORK ON DEVELOPING HYBRID WHEAT AT THE SADOVO STATION

The work on the use of cytoplasmic male sterility in developing hybrid wheat in Bulgaria started in 1964 at the Wheat Institute in General Toshevo (POPOV—GOTSOV 1967a, b) and in 1967 at the "K. Malkov" Agricultural Experimental Station, Sadovo, Plovdiv district.

As a result of the studies carried out so far on the heterosis phenomena in wheat, a number of problems have been elucidated concerning the combining ability of some perspective Bulgarian and foreign varieties of soft winter wheat, as well as the manifestation of the heterosis effect in F_1 on a fertile basis (POPOV—GOTSOV 1967b, 1968c, POPOV—PAVLOV 1967, 1970, POPOV—GOTSOV 1970, POPOV—STANKOV 1972).

Besides, it was established that the cytoplasm of *T. timopheevi* ranges among the most appropriate ones for the development of sterile analogues of the varieties of soft winter wheat (POPOV—GOTSOV 1968c, POPOV *et al.* 1973).

Most of the studies however, deal with the problems of the development of effective fertility restorers, as well as those of free pollination in wheat (POPOV—GOTSOV 1968a, 1969b, 1970, 1971a, 1971b, POPOV *et al.* 1971).

During the last few years research on the development of hybrid wheat under the conditions of South Bulgaria proceeded in the following directions:

1. Development of sterile analogues of soft winter wheat by use of the cytoplasm of *T. timopheevi.*

The satiation was continued with these lines after the back-cross type of the sterile forms possessing cytoplasm of *T. timopheevi* with the most perspective Bulgarian and foreign varieties. Althogether 134 varieties and lines of soft winter wheat were transferred on a sterile basis. Finally, sterile analogues have already been developed from a great number of varieties and lines of Bulgarian and foreign selections, including the most perspective ones — Bezostaya 1, Avrora, Kavkaz, Hebros, Skorospelka 35, etc. The newest high-producing varieties and lines Sadovo 1, Burgas 2, Bezostaya 2, etc., were also included in the program for the development of sterile analogues.

The satiation was carried out mostly by means of the modified method of forced pollination of 4—5 florets by one fully mature stamen. At the same time, the comparative testing of the following four methods of pollination was also continued;

1st method — limited-free pollination by wheat ears;

2nd method — pollination by means of grinding an ear of the pollinator variety over an ear of the sterile form of which 1/3 of the florets had been removed;

3rd method — forced pollination — the classical method (1 stamen per 1 floret).

4th method — forced pollination — a modified method (1 stamen per 4—5 florets, through rubbing).

This year again the results pointed out the effectivity of forced pollination after the classical and the modified method (3rd and 4th method — 53.7% and 63.1%, respectively) in comparison with the other two methods (1st and 2nd method — 22.5% and 12.1%, respectively).

Twelve sterile analogues having good prospects for development under the conditions of this country were included for reproduction. The seed set with MS analogues varied from 9.1% to 71.4%. Comparatively high seed set was obtained from MS Skorospelka 35—71.4%, MS Hebros —66.3%, etc.

Unsatisfactory seed set was mainly due to the low pollen density of the respective pollinators.

2. Development of fertility restorers — testing the restoring and combining ability and reproduction of the most perspective R lines.

The basic trend in our work with hybrid wheat was the development of our own full fertility restorers of a high combining ability, possessing the productivity of the new varieties. For this purpose we chiefly used a modified pedigree method — the selection being conducted in F_2 and the testing of the new R lines in F_3. In this way 170 R lines were selected, which are a product of the breeding work carried out and possess almost full restoring ability. After their evaluation in terms of productivity and other useful agronomic qualities, 39 R lines were selected among them, their restoring ability ranging from 71.6% to 93.9%. They originate from the following crosses: R_4* × Bezostaya 1^1—7 lines, R_4 × Palmares ^{1}—13 lines, R_4 × 19—16^1—7 lines, R_{13}** × Bezostaya 1^1—3 lines, R_{13} × Kavkaz^1 — 2 lines, etc.

The most promising 10 R lines were planted on an isolated plot for hybrid seed production, with 6 MS analogues.

The study on the restoring ability of R_4 and R_{13} at forced and free pollination was also continued. Thus, from the examined 45 test crosses with Primepi, the restoring ability as regards the fertile Primepi at self- pollination varied within the 62.1% — 99.5% range, and at free pollination — within the 80.5%97.7% range.

With the investigated 52 test crosses with Palmares, the restoring ability regarding the fertile Palmares variety ranged from 20.1% to 64.1% at forced pollination, and from 39.3% to 93.9% at free pollination.

The results obtained show that the variety Primepi is one of the best fertility restorers.

The following lines of high restoring ability were developed by means of selection: 26 lines from R_4, 7 lines from R_{13}, etc.

3. Testing the combining ability of various varieties and lines and establishing the most appropriate hybrid combinations on a fertile and sterile basis.

Testing of hybrids on a fertile basis was conducted with the participation of the Primepi and Palmares varieties. A total of 36 crosses obtained on a fertile basis in 2—3 replications was tested on an area of 0.4 to 5.2 m^2 at 20/5 cm spacing. Out of the 36 crosses only 11 demonstrated high hybrid vigour towards the standard variety Bezostaya 1. Highest hybrid vigour was observed with the following crosses: Kavkaz × Primepi — 160.1%, Avrora × Primepi — 160.1%, Avrora × Palmares — 127.2%, Hebros × Palmares — 123.3%, etc. The results obtained show the possibilities for using the two varieties as direct fertility restorers in some hybrid combinations of high producing varieties.

Besides these, 134 hybrid combinations were studied on a sterile basis, 68 of them being obtained with the participation of the Primepi variety, and 66 with the participation of Palmares.

Each testing was effected in two replications of 4 m^2, parallelly with the parent varieties and the standard Bezostaya 1 variety.

Of 68 crosses, formed with the participation of Primeri, 26 were more productive as compared to the standard variety Bezostaya 1. Especially good in this respect were: MS Fertődi^3 × R_4 — 153.4%, MS Bezostaya 1^3 × R_4 — 151.9%, MS 19—16^4 × R^4 — 143.9%, etc.

The behaviour of the remaining 66 crosses, obtained with the participation of the other French variety — Palmares, was different. Only 10 combinations were superior to the standard Bezostaya 1 variety (from 100.9 to 113.1%). That is why the Palmares variety is of less interest for direct use as a fertility restorer. Therefore, only the R lines selected from it can be used, but not the variety itself as a population.

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Prepared at the "K. Malkov" Agricultural Experimental Station, Sadovo, Plovdiv district.

P. Popov, I. Stankov

R_4* Primepi: R_{13}** —*t 8* Palmares

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HEBRICIDES INCREASING THE PROTEIN CONTENT OF LUPIN

As regards the effect of herbicide treatments on the protein content of legumes, Cooke (1955) showed increases in the soluble and total nitrogen content of legumes treated with Monuron (3-p-chloro-phenyl 1, 1-dimethyl urea). These increases were accompanied by decreases in the sugars and pectins. No increases in plant growth were noted.

According to Ries—Larsen—Kentworthy (1963) Simazine (2-chloro-4,6-bis (ethylamino) -S-triazine) mixed with Amitrole-T (3-amino-1,2,4-triazine plus ammonium thiocyanate) treatments of peach and apple trees increased the leaf nitrogen content. Freney (1965) reported that 1.5 ppm Simazine increased the uptake of nitrogen in maize only, when additional nitrogen was applied to the soil in a glasshouse experiment. Ries—Gast (1965) also proved that Simazine and hidroxy Simazine increased the N content of maize. Karnatz (1964) found that fruit trees have a higher level of N content in their leaves due to Simazine.

CONNER—WHITE (1968) stated that 0.8 ppm Simazine increased foliar N concentration by 23.81 per cent which was equivalent to trebling the amount. TWEEDY *et al.* (1971) reported that 1.12 kg/ha Simazine increased the yield and crude protein content in sorghum grain when the plants were under N stress.

We have also found herbicides increasing the N content (crude protein) and water-soluble protein content of the top of *Lupinus albus* and *L. luteus* during our small-plot field experiments (1970, 1971, 1972) carried out in a slightly acid forest soil of Nyírség (Gyulatanya) in Hungary, using 110 herbicides belonging to 28 different chemical groups (KECSKÉS—BORBÉLY—BORBÉLY—ELEK 1971, BORBÉLY—BORBÉLY—ELEK—KECSKÉS 1972, KECSKÉS—ELEK—BORBÉLY—BORBÉLY 1972).

In two years (1970, 1971) we analysed the protein content of the lupin, seed samples of 15 herbicide treatments, and samples were taken for analysis from green plants of white and yellow lupin (80 treatments). The crude protein was calculated on the basis of the total N content determined by the Kjeldahl method and the water-soluble protein was analysed after trichloroacetic-acid precipitation.

The samples were taken from the white and yellow lupin plants of 45 and 35 altogether 80 treatments (sprayed pre-emergently) after the phenological observations, the original aim being to decide whether the herbicide treatments decreased the protein content of the lupin. The samples were collected at flowering and the analyses were made from the average samples of 2—2 duplicates.

9 herbicides (belonging to triazine, urea, phenoxy and uracil derivatives) proved to be protein-increasing.

As a preliminary report we should like to draw attention to four of them: Gesatop 50 (2-chloro-4,6-bis-ethylamino 1,3,5-triazine), A 2591 (4-ethylamino-2s-butylamino-6-methoxy-s-triazine), Camparol (A 1803) (Prometryne+Simazine) and Venzar (3-cyclohexyl--5,6-trimethyleneuracil).

The protein content of the seed samples taken from herbicide- treated lupin plots did not show significant differences in any year compared to the hoed control ones (Table 1).

As the data show, the water- soluble protein of *L. albus* was increased by the herbicides Gesatop 50, Venzar, while A 2591, as well as the combined herbicide Camparol was nearly the same as the control. The crude protein was more than 10 per cent higher in all the white lupin top samples (Table 2).

Table 1

Herbicide treatments increasing the protein content of Lupinus albus (1971)

Treatment*	Crude protein %		Water soluble protein %	
	in dry weight	compared to the hoed	in dry weight	compared to the hoed
Hoed control	22.68	100.0	18.72	100.0
Gesatop 50	25.15	110.8	21.70	116.0
A 2591	25.15	110.8	18.90	101.0
Camparol 1803	25.68	113.2	18.90	101.0
Venzar	25.68	113.2	21.70	116.0

* 3.5 kg/ha

Table 2

Herbicide treatments increasing the protein content of Lupinus luteus
(1973)

Treatment*	Crude protein %		Water soluble protein%	
	in dry weight	compared to the hoed	in dry weight	compared to the hoed
Hoed control	15.88	100.0	11.23	100.0
Gesatop 50 (2.5 kg/ha)	18.59	117.1	13.71	122.1
A 2591	15.88	100.0	15.04	133.9
Camparol 1803	17.50	110.2	11.17	99.5
Venzar	17.50	110.2	10.09	89.8

* 3.5 kg/ha

In the case of *L. luteus* only the Venzar decreased the water-soluble protein content the Camparol was practically the same as the control and the A 2591 increased it by more than 30 per cent and the Gesatop treatment by 22.1 per cent. The crude protein content of yellow lupin plants was more than 10 per cent higher in every treatment of Table 2, except the A 2591 treatment which was at the control level.

We have to remark also that in the course of our selective investigations these herbicides proved to be not unfavourable from the point of view of the weed control of white or yellow lupin (on the basis of the grain yield, the root nodulation of plants, as well as the number-weight and the occurrence of the dominant species of weeds and also according to our microbiological tests, these are not very toxic at all to the rhizobia and especially to the strains of *Rhizobium leguminosarum* (Kecskés—Borbély—Borbély—Elek 1971, Kecskés—Elek—Borbély—Borbély 1972).

The data resulting from field experiments using the herbicide doses of agricultural practice seem to prove that (beside their weed-control effect and often their harmful effect on the cultivated plants) there could be herbicides which have some favourable effect on lupin too.

Although their incorporation into the plant protein has not yet been studied, the question is, that when it is built into the plants, is it also dangerous to the animal? This is an open question but would be very important for the protein-demanding world if the protein content of the protein-rich legumes were increased by herbicides.

*

Prepared at the Research Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, Agrotechnical Research Institute of Nyírség, Nyíregyháza.

É. Elek, I. Borbély, F. Borbély, M. Kecskés

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INDUCED AUTOPOLYPLOIDY IN SESAME (SESAMUM ORIENTALE L.)

The induction of autoploidy in sesame was attempted in India and in Venezuela (RICHHARIA—PERSAI 1940, KOBAYASHI—SHIMAMURA 1952, SRIVASTAVA 1956, LANGHAM 1942, MAZZANI—ZERPA 1953). The immediate effects of colchicine differed with the treated plant part and with the method of treatment. There was no general agreement on the effect of chromosome doubling on the morphology and the productivity of the plants.

On the other hand, the Indian workers succeeded in transferring resistance to phylloidy and to the web-worm (*Antigastra catalaunalis*) from *Sesamum prostratum* ($2n = 32$) to *S. orientale* ($2n = 26$) through the artificial amphidiploid—*S. indicatum*, $2n = 58$ (RAMANUJAM 1942, 1944, RAGAHAVAN—KRISHNAMURTHY 1947, RAMANUJAM—JOSHI 1951).

The aim of the present study is to test the effectivness of two methods of colchicine treatmen in the induction of autopolyploidy and to study the effect of chromosome doubling on growth and productivity of a local sesame variety.

The colchicine treatments started in June, 1960 and the variety used was "Mass Selection Early Resistant White" (a local Sudanese variety). The methods of treatment were as follows.

1) Seed-immersion method. The seeds were soaked in tap water for 12 hours, washed with distilled water and then rinsed in colchicine solutions of different concentrations for different durations. The seeds were washed with the prescribed solution before being rinsed in order to avoid lowering the concentration. The concentrations of colchicine ranged from 0.04% to 0.5% and the durations from two hours to five days. After the treatment the seeds were thoroughly washed to remove traces of colchicine. The treated seeds were either germinated in Petri dishes covered with wet filter paper, or sown in pots.

2) Shoot-tip treatment. The shoot-tips of 5—7- day- old seedlings were treated either with drops of aqueous colchicine solution or with agar-colchicine mixture. The application

was performed at 8 a.m. and at 5 p.m. for a number of successive days. The seedlings were kept in green-house. The colchicine solution treatments ranged from 0.05% to 0.8% for 3, 4 or 5 days. The agar—colchicine mixture was prepared by mixing equal amounts of colchicine solution with 1.0% agar and then applied to the shoot-tips after it cooled off. The concentrations of colchicine ranged from 0.05% to 0.5% were applied for 5 consecutive days.

The characteristics of the treated plants were followed up to the flowering stage when it was possible to detect and identify the autotetraploid plants. Further data were recorded on both autotetraploid and diploid plants with regard to pollen-grain size, fruit size, seed size and the number of seeds per pod.

The diploid plants of *S. orientale* have 13 pairs of chromosomes, which are tiny and difficult to count. Thus chromosome countings to identify individual tetraploid plants were not carried out. The main criterion for the detection of autotetraploid plants, during the course of this investigation, was the size of pollen grains. Many investigators found no exception to the rule that pollen-grain size increases with chromosome doubling (BLAKESLEE—AVERY 1937, STEBBINS 1950, DERMEN 1954).

The following observations and measurements were recorded on the progeny of the autotetraploid plants:

Size of successive leaves, number of days to first flowering, node number at which the first floral bud appeared, number of branches per plant, size of the flower, size of the pollen grains, pollen sterility, number of pods per plant, number of seeds per pod, number of seeds per 0.5 g and oil content.

1) *The seed-immersion method*

In the first esperiment, lots of 10 seeds were treated with colchicine solutions of different concentrations and for different durations, and then germinated in Petri dishes, covered with blotting papers. The effect of treatments was clear after six days. The rate of growth was greatly retarded, especially when using concentration higher than 0.06 per cent. The upper part of the hypocotyl was swollen in the case of concentrations up to 0.06 per cent while concentrations above that caused the swelling of the entire hypocotyl and stunting of the root (Fig. 1). The extent of both types of swelling was proportional to the concentration and duration of the treatment. The cotyledonary leaves of the treated seedlings were larger, rounder, darker green and thicker than those of the control ones.

In the second experiment, lots of 100 seeds treated with different concentrations for various durations were sown in pots. The emerged seedlings which persisted until maturity were counted as survivals. The percentage of surviving seedlings varied mainly with the concentration rather than with the duration (up to 12 hours) of treatment. A marked drop in the percentage of survivals occurred at durations longer than 12 hours. The concentration 0.2 per cent for 12 hours gave 49 per cent survivals, while the same concentration for 24 hours resulted in only 6 per cent.

The number of autotetraploid plants was very small in all ranges of treatments. Only 27 autotetraploid plants from 2400 treated seeds were obtained, i.e. 1.1 per cent. The concentration 0.04 per cent for 2, 4, 6 and 12 hours gave no autotetraploid plants. The concentration 0.08 per cent for 4 hours and for 12 hours resulted in 4 autotetraploid plants each. The concentration 0.2 per cent resulted only in one plant for each duration of one day, two days, three days and five days. The concentration 0.5 per cent for 5 days resulted also in one autotetraploid plant.

These findings indicate that the method of seed immersion is not efficient for inducing polyploidy in sesame.

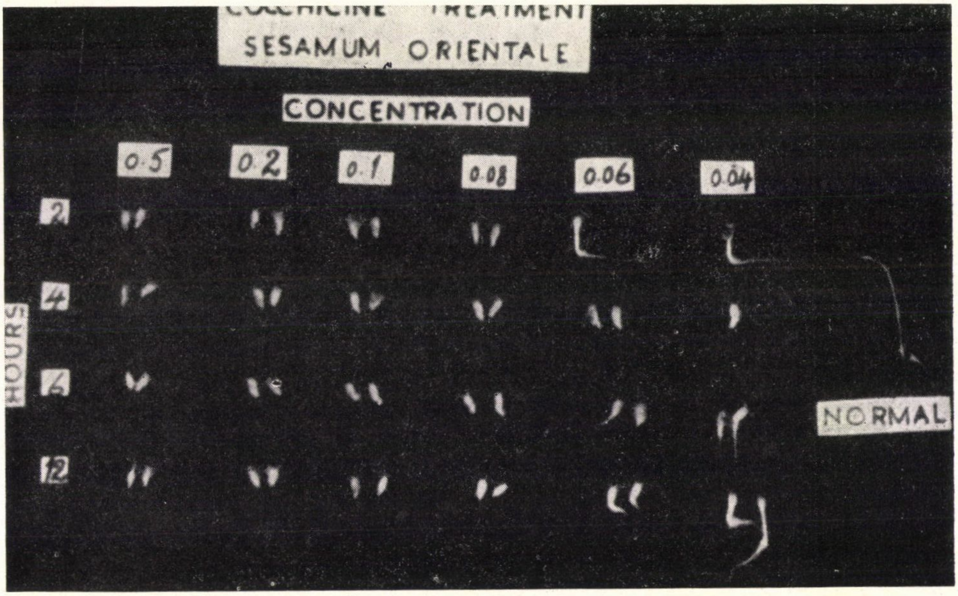

Fig. 1. Progressive swelling of hypocotyl and root stunting following seed immersion in colchicine solutions

2) *The shoot-tip treatment*

The application of both aqueous colchicine solution and agar-colchicine mixtures to the shoot-tips of 5 to 7- day- old seedlings gave nearly similar results.

The outcome of the treatments with aqueous solutions of different concentrations and for various durations is presented in Table 1. It is clear from this table that the higher the

Table 1

The results of shoot-tip treatment with aqueous solutions of colchicine of different concentrations and for various durations. Lots of 100 seedlings were treated

Concentration %	Duration					
	3 days		4 days		5 days	
	Survivals	Autotetraploids	Survivals	Autotetraploids	Survivals	Autotetraploids
0.05	—	—	65	8	55	8
0.10	—	—	68	15	38	20
0.20	45	12	53	34	35	25
0.30	—	—	43	23	30	18
0.40	—	—	40	10	28	15
0.50	—	—	28	13	28	15
0.80	—	—	30	8	—	—

Control survivals = 93 polyploids = 0

concentration or the longer the duration of treatment the greater was the number of dead seedlings. The percentage of autotetraploid plants for the duration of 4 and 5 days was more or less similar, but higher than the percentage at the duration of 3 days. The highest percentage of autotetraploid plants was obtained by treating the seedlings with 0.2 per cent aqueous solution of colchicine either for 4 or 5 days. This percentage decreased at concentrations lower or higher than 0.2 per cent.

The outcome of the treatments with agar-colchicine mixtures is shown in Table 2.

Table 2

The results of shoot- tip treatment with colchicine — agar mixtures of different contentrations for 5 days

Concentration %	No. of treated seedlings	No. of seedlings obtained	No. of autotetraploid plants	% of autotetraploidy
0.05	50	42	4	8
0.10	50	39	7	14
0.20	50	38	15	30
0.30	50	36	10	20
0.40	50	30	4	8
0.50	50	31	3	6

The percentage of survivals was proportional to the concentration. It was 84 per cent after **the treatment** with 0.05 per cent, while it was only 62 per cent at the concentration 0.5 per **cent.** The concentration 0.2 per cent of agar—colchicine mixture gave the highest percentage of **autotetraploid** plants.

3) *The characteristics of the treated plants and the resulting autotetraploids*

Shoot emergence. This first noticeable effect of shoot-tip application was the delay in shoot emergence from the treated terminal bud. The growth was even stopped for a few days,

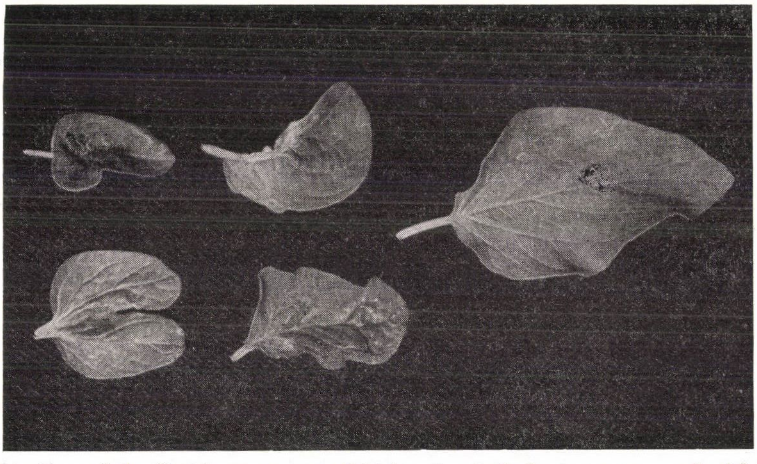

Fig. 2. Distortion of the first leaves on a colchicine- treated plant as compared with that of an untreated plant (top leaf)

then resumed slowly. Some shoot-tips showed burning effect, few died out and the majority formed callus-like tissue from which new shoots developed after a number of days.

Leaf characters. The first few leaves emerging from the treated buds were dark green, crinkled and some of them distorted and with asymmetrical lamina (Fig. 2). The leaves which developed higher up on the treated plants also showed marked differences from the corresponding leaves on the untreated plants. On the 4th, 5th and 6th nodes the diploid leaves were usually 3-partite and yellowish- green. The corresponding leaves of the tetraploid plants were simple, broader and of darker green colour.

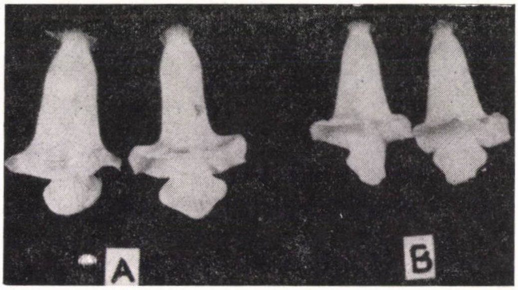

Fig. 3. Flowers of an autotetraploid (A) and a diploid plant (B)

Growth characters. The autotetraploid plants, though shorter at an early stage, gradually became equal or even taller and more robust than the diploid plants at later stages of development. The autotetraploid plants were on the average about 21 days later in flowering than the diploid ones. Likewise, the flowering period of the autotetraploid plants was more prolonged than that of the diploid plants.

The size of the flower. The flowers of the autotetraploid plants were larger than those of diploids (Fig. 3). The calyx segments were longer, broader and more hairy. The corolla tube was larger and thicker, and the ovary was broader and surmounted by a thicker, more or less shorter style with larger stigmatic lobes.

The size of the pollen grains. The pollen grains of the autotetraploid plants were bigger than those of the diploid ones and it was always easy to distinguish them with microscopical examination. The average size of the tetraploid pollen grains was 82.8 × 65.8 micron, while the average size of the diploid pollen grains was 68 × 49.3 micron (average of 500 pollen grains each). In other words, chromosome doubling resulted in about 64 per cent increase in pollen-grain size.

The size of the pods. The autotetraploid capsules were shorter and broader than the diploid ones. The average size was 17 × 8 mm for the tetraploids and 23 × 7 mm for the diploids (average of 500 pods each). Longitudinal median cuts in the capsules showed that the placenta and the developing ovules were enlarged in the autotetraploid (Fig.4).

The seeds. It was evident that the autotetraploid pods contained fewer seeds than the diploid ones. Table 3 shows the pod contents of both types of plants. It is clear that the total number of ovules in the ovary was much less in the autotetraploid pods, and that a considerable number of ovules has not been fertilized. The size of the seeds, on the other hand, was greatly increased in the tetraploids (Fig. 5). The average number of polyploid seeds per 0.5 g was 143, while the average number of diploid seeds was 235. It could be stated, therefore, that polyploidy resulted in about 60 per cent increase in seed size.

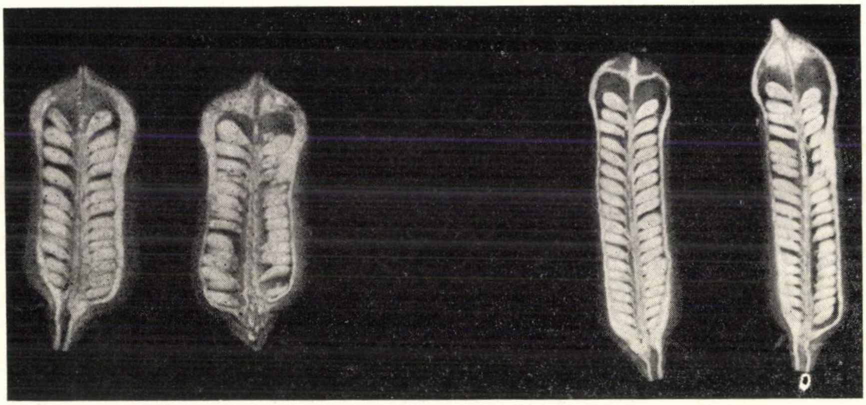

Fig. 4. Capsules of an autotetraploid (left) and a diploid plant (right)

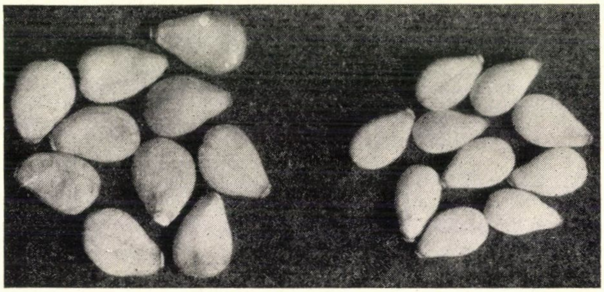

Fig. 5. Autotetraploid seeds (left) and diploid ones (right)

4) *The autotetraploid progeny*

Seed germination of both autotetraploid and diploid types was studied. Table 4 gives the rate and total germination percentage of each.

The germination of the autotetraploid seeds started one day later than that of the diploid ones. The maximum emergence per day was 150 seedlings for the diploids, occurring 5 days after sowing, while in the autotetraploids the maximum daily emergence was 82 seedlings occurring 7 days after sowing. The total percentage of germination was 70 in the autotetraploid

Table 3

The pod contents of diploid and tetraploid plants. Average of 500 diploid and 250 tetraploid pods

Type of plant	Fertilized ovules	Undeveloped ovules	Total	Sterility %
Diploid	49.0	2.5	51.5	4.8
Tetraploid	24.7	9.8	34.5	28.4

Table 4

The number of seeds germinating daily from 300 seeds of autotetraploid and diploid types sown in pots on 11. 4. 1961

Date	Number of seedlings	
	Diploid	Autotetraploid
13/4	0	0
15/4	24	0
16/4	150	30
17/4	20	18
18/4	66	82
19/4	25	52
20/4	0	17
21/4	0	14
22/4	0	0
Total	285	213
Percentage	95	70

seeds as compared with 95 in the diploid ones. The autotetraploid seedlings were characterized by thicker hypocotyl, larger, thicker and darker green cotyledonary leaves than the diploid ones.

Each of the first 3 successive pairs of leaves appeared two days earlier in the diploids than in the autotetraploids, while the 7th pair was unfolded the same day in both. This indicated a slower growth rate of the autotetraploid plants at the beginning, but it was later increased. The leaves of the autotetraploids were characteristically simple up to the 8th or 9th nodes, while in the diploid plants only the first four pairs of leaves were simple and the upper leaves up to about the 8th node had 3-partite lamina.

The flowering behaviour showed significant differences between the diploid and autotetraploid plants. Flowering started in the autotetraploid plants in general after 63 days, and the first flower appeared on an average node number of 9, while in the diploids flowering started in general after 50 days, and the first flower developed on an average node number of 8. This indicated a delay of about two weeks in the flowering of the autotetraploid plants. The flowers of the autotetraploid plants were larger than those of the diploids, averaging 26.6 × 15.4 mm in the former and 24.5 × 12.6 mm in the latter.

The size of the pollen grains showed a significant difference between the autotetraploids and the diploids — being larger in the former. The average size of the autotetraploid pollen grains was 84.2 × 63.4 microns, while that of the diploids was 71.6 × 49.0 microns (average of 500 pollen grains each). The percentage of aborted pollen counted in a total of 250 was 23.9% in the autotetraploids, and only 2.6% in the diploids. The aborted pollen grains were distinguished by their small size and uneven surface.

The number of mature pods per plant was found to be on the average 75 in diploids as compared with 54 in the autotetraploids. This was largely due to the shedding of a number of tetraploid floral buds at an early stage. The autotetraploid plants were characterized by larger number of undeveloped ovules and consequently smaller number of seeds per pod than the diploids. Table 5 gives the pod contents of both types of plants.

Table 5

Sterility per capsule of diploids and autotetraploid; average of 100 pods each

Type of plant	Fertilized ovules	Undeveloped ovules	Total	Sterility %
Diploid	52.3	3.5	55.9	6.9
Autotetraploid	22.6	10.1	32.7	30.9

It is evident that the total number of ovules per ovary was reduced as a result of chromosome doubling, being 32.7 in the autotetraploid and 55.9 in the diploids. However, the size of the seeds was greatly increased in the tetraploid pods. The average number of autotetraploid seeds per 0.5 g was 150, while that of the diploid seeds was 234, indicating 56 per cent increase in seed size. The oil content was slightly affected by chromosome doubling, being 48.3 per cent in the diploids and 44.3 per cent in the autotetraploids.

The above account of the autotetraploid progeny indicates that they were similar to their parents. In other words, the autotetraploid plants bred true.

The present study indicates that colchicine application to the shoot-tips of sesame seedlings was more efficient than seed- immersion treatment for the inducation of polyploidy. As high as 30 per cent of autotetraploid sesame plants were produced using the former method, while only 1 per cent was obtained by the latter. It seems that the treatment of seeds resulted in overtreatment of the roots, since the root cells are more actively dividing than the epicotyl cells at the early stages of germination. As a result, the chromosome number of the root cells may become more than double and thus the division may be arrested- leading to stunted roots and consequently to the death of the seedling.

The first noticeable effect of shoot-tip treatment was a retardation in shoot emergence. The first emerging leaves were distorted and darker green that those of the diploids. The autotetraploids plants showed slower growth rate and flowering started about 21 days later than in the diploid plants.

The size of the pollen grains was the main criterion for detecting autotetraploid plants. Pollen grains from the first flowers were used for this purpose. This seemed necessary since it has been reported that there is a considerable difference in pollen-grain size between the plants just beginning to flower and those at the end of the flowering period.

The autotetraploid plants were less productive than their diploid progenitors. They were characterized by a higher number of aborted pollen grains and reduced number of pods per plant. The pods were shorter and broader, and contained less number of seeds than the diploid ones. The reduction in seed set (being about 28 per cent) could be considered small in comparison with that of induced autotetraploids in some other crop plants. KOSTOFF (1940) found that the shorter the chromosomes, the lower the frequency of chiasmata and quadrivalent formation, and consequently, the higher is the fertility in the autotetraploid. Thus a fairly high fertility seems to have resulted in sesame. However, a part of the reduction in seed set is due to the reduction in the number of ovules per ovary.

The seeds of autotetraploid sesame were about 60% bigger than the diploid ones. This increase in size could be attributed either to the direct effect of chromosome doubling, or to the fact that they were few in number (thus received abundant food material), or to a combination of both factors.

Certain findings of the present investigation seem to be in accordance with some previous reports and disagree with others. With regard to the number of pods per plant, LANGHAM

(1942) and SRIVASTAVA (1956) indicated that there was no effect of autotetraploidy on seed set. The increase in seed size of autotetraploid sesame was reported by LANGHAM (1942), KOBAYASHI—SHIMAMURA (1952) and SRIVASTAVA (1956). On the other hand, MAZZANI—ZERPA (1953) claimed that the seeds were shrivelled and with lower oil content.

A comparison in net oil yield between the diploid and the autotetraploid sesame plants could be made, using the following formula:

$$\text{No. of pods/plant} \times \text{No. of seeds/pod} \times \text{weight of 100 seeds} \times \frac{\text{oil content}}{100}$$

The relative oil yield of a diploid and an autotetraploid plant could be roughly estimated as follows:

$$\text{Diploid} = \frac{75.3 \times 22.3 \times 0.21 \times 48.3}{100 \times 100} = 3.99 \text{ g of oil}$$

$$\text{Autotetraploid} = \frac{54.4 \times 22.6 \times 0.33 \times 44.3}{100 \times 100} = 1.80 \text{ g of oil}$$

Therefore, the reduction in the net oil yield resulting from chormosome doubling is 54.88 per cent.

The only merit of autotetraploid sesame seems to be the marked increase in seed size, which was accompanied by reduction of other productive factors. If characters such as number of pods per plant, seed set and oil content could be improved by selection, either straight or following crosses between autoteraploids derived from different varieties, higher oil yield per plant would be achieved and autotetraploid sesame would prove of economic value.

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Prepared at the Faculty of Agriculture, University of Khartoum, Khartoum.

M. O. KHIDIR, M. A. ALI

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THE EFFECTS OF NITROGEN AND POTASSIUM FERTILIZERS ON MAIZE YIELD IN CALCAREOUS SANDY SOILS

A considerable part of Hungarian arable lands consists of different types of sandy soils. More than half of them are calcareous sands and they occupy 20% of the cultivated area. As regards their occurrence, the largest part of calcareous sands can be found in the region between the Danube and Tisza rivers. Several decades ago, when the fertilizer level used was relatively low in this country, low- requirement plant cultures were grown primarily in these soils. Nowadays the application of large fertilizer doses makes it possible to grow crops like maize on otherwise unfavourable sandy soils. There are many authors dealing with the application of fertilizers on sandy soils in Hungary (LŐRINCZ 1969, LÁNG 1971, BAUER 1964, LÁNG 1973).

Most of the literature avaiable is connected with the yield- increasing effect of nitrogen fertilizers and relatively few data can be found with regard to phosphorus and potassium fertilizers (ANDERSON 1959, STANGEL 1965, GYŐRFFY 1962, MRS. LATKOVICS 1964).

According to the experimental results found in the literature, nitrogen has primary importance among the fertilizers, though the simultaneous application of potassium and phosphorus is necessary for reaching the highest possible yields.

Our task was to study how different levels of nitrogen and potassium fertilizers affect the maize yields in one of the most typical areas of the sand-ridge between the rivers Danube and Tisza. The rainfall conditions of the region are shown in Table 1.

It can be seen from this Table that the amount of annual rainfall varied between 432—718 mm and every year it strongly deviated from the 50 years'n mean. On the basis of the data, it can be established that more than 50% of the rainfall occurs in the vegetation period.

The data of the soil analysis of the experimental area are shown in Table 2. On the basis of the data presented it can be realized that the pH of the soils in the experimental area was about 8. The $CaCO_3$ content was high, while the nutrient content with the exception of phosporus was low. The phosphorus content, compared to the other two nutrient elements, was rather high and this was taken into consideration when setting up the experiment.

The experiment was carried out in 5 replications and 16 treatments were applied in the years 1968—1972. The size of each experimental plot was 72 m². With the exception of the control without any fertilizers, 50 kg/ha P_2O_5 superphosphate were applied in all treatments. The doses of nitrogen and potassium fertilizers were determined at 4 levels, i.e. 40, 80, 120 and 160 kg/ha N/K_2O. The determination of certain fertilizer doses was done by using partly the present application and partly considering the long-range plans of future fertilization.

The agrotechnical procedures applied in the experimental field were identical with the usual ones developed in the area. Spring ploughing was applied and sowing and harvesting

Table 1

The monthly rainfall formation and deviation from the 50-year average

Period	1968		1969		1970		1971		1972	
	mm.	deviation	mm.	deviation	mm.	deviation	mm.	deviation	mm.	deviation
January	18	−12	32	+ 2	67	+37	65	+35	19	−11
February	23	− 8	130	+99	57	+26	12	−19	37	+ 6
March	24	− 9	35	+ 2	80	+47	39	+ 6	12	−21
April	45	− 3	25	−23	59	+11	38	−10	38	−10
May	22	−31	21	−32	43	−10	68	+15	56	+30
June	20	−45	164	+99	36	−29	30	−35	84	+19
July	51	+ 3	35	−13	49	+ 1	43	− 5	79	+31
August	78	+34	44	—	86	+42	15	−29	118	+74
September	82	+38	26	−18	21	−23	46	+ 2	20	−24
October	3	−45	18	−30	20	−28	20	−28	65	+17
November	51	+ 2	56	+ 7	24	−25	40	− 9	69	+20
December	15	−22	132	+95	42	+ 5	36	− 1	27	−10
Annual total	432	−98	718	+188	584	+54	452	−78	624	+94
vegetation period During (Apr.—Sept.)	298	− 4	315	+13	294	− 8	224	−70	395	+93

Table 2

Main characteristics of experimental soil
(in 0—30 cm ploughed layer.)

Sample (average)	pH		$CaCO_3$ %	hy	K_A	Humus %
	H_2O	KCL				
1st rep.	8.1	7.7	10.2	0.87	28	1.64
2nd rep.	8.0	7.7	9.2	0.95	29	1.62
3rd rep.	8.1	7.8	10.5	0.98	28	1.52
4th rep.	7.9	7.8	14.2	0.62	27	1.31
5th rep.	7.8	7.6	15.6	1.03	29	1.46

Sample (average)	Total nitrogen mg.%	P_2O_5 mg.%	AL-soluble K_2O mg.%
1 st rep.	120	37.2	21.2
2 nd rep.	108	39.8	20.8
3 rd rep.	117	39.7	19.8
4 th rep.	92	16.3	13.4
5 th rep.	95	21.3	12.1

were carried out manually to ensure the required plant population. In 1968—1969 Mv-1 hybrid maize was sown, in 1970 Mv-40 and in 1971—1972 Mv-431 with 31 thousand/ha plant population in our experiments. Due to the heterogeneity and for precision in the assessment of sandy soils the experiment was set up in balanced lattice square arrangement. The evaluation was carried out with analysis of variance, using row and column correction.

For the pooled statistical analysis of the crop data of five years, mean data — related to each plot — were calculated and mathematical evaluation was carried out with these.

Table 3

Crop results
(in 5- year average)

Treatment	Crop 86% dry matter		
	q/ha	D	%
Control	35.1	—	100
$N_{40}P_{50}K_{40}$	40.5	5.4	116
$N_{40}P_{50}K_{80}$	42.0	7.7	122
$N_{40}P_{50}K_{120}$	43.9	8.8	125
$N_{40}P_{50}K_{160}$	42.7	7.6	121
$N_{80}P_{50}K_{40}$	43.7	8.6	124
$N_{80}P_{50}K_{80}$	47.9	12.8	136
$N_{80}P_{50}K_{120}$	46.9	11.8	133
$N_{80}P_{50}K_{160}$	47.8	12.7	136
$N_{120}P_{50}K_{40}$	46.6	11.5	133
$N_{120}P_{50}K_{80}$	46.7	11.6	133
$N_{120}P_{50}K_{120}$	49.6	14.5	141
$N_{120}P_{50}K_{160}$	49.1	14.0	140
$N_{160}P_{50}K_{40}$	46.6	11.5	133
$N_{160}P_{50}K_{80}$	45.8	10.7	131
$N_{160}P_{50}K_{120}$	48.0	12.9	137
$N_{160}P_{50}K_{160}$	47.8	12.7	136
LSD 5%		6.1	18

Table 4

The effect of N- and K — fertilization on the crop of maize
(in 5-year average)

Treatment	Grain yield 86% dry matter q/ha						
	K_{40}	K_{80}	K_{120}	K_{160}	LSD 5%	Average	%
N_{40}	40.5	42.8	43.9	42.7	4.4	42.5	100
N_{80}	43.7	47.9	46.9	47.8		46.6	109.6
N_{120}	46.6	46.7	49.6	49.1		48.0	112.9
N_{160}	46.6	45.8	48.0	47.8		47.0	110.6
LSD. 5%			4.4			2.2	5.2
Average	44.4	45.8	47.1	46.8	2.2	46.0	
%	100.0	103.1	106.1	105.4	4.9		

The results of the experiments are reported in Table 3. The two- dimensional corrected table of results can be found in Table 4 without the checks showing the mean nitrogen and potassium nutrient doses for each level of fertilizer application.

The data relating to analysis of variance are given in Table 5. It can be seen that both the nitrogen and potassium fertilizer effects were significant, though the probability level deviated. N proved to be effective already at 0.1% probability level, while K only at 10% probability level. The quadratic component within the N effect is significant, while the linear component is highly so. In the case of potassium the linear component was slightly significant, and the square component was not significant at all.

Investigating the effect of the different nitrogen treatments it could be established that the fertilizer effect was of increasing tendency and resulted in reliable crop surpluses in all

Table 5

Data on the analysis of variance

Factor	SQ	FG	MQ	F
Treatment	2535.81	15	169.05**	2.96
N	1816.17	3	605.39***	10.61
L	1174.89	1	1174.89***	20.59
Q	640.85	1	640.85**	11.23
Rest.	0.43	1	0.43	
K	475.20	3	158.40+	2.77
L	391.88	1	391.88*	6.87
Q	73.53	1	73.53	1.29
Rest.	9.79	1	9.79	
N×K	244.44	9	27.16	
L×L	2.76	1	2.76	
Rest.	241.68	8	30.21	
Error		30	57.07	

Level of significance: × × P = 1%, × P = 5%, + P = 10%.

Table 6

Grain-yield surplus for 1 kg N and K_2O

Applied fertilizer	Grain-yield surplus 86% dry matter
N_0-N_{40}	13.56
$N_{40}-N_{80}$	19.32
$N_{80}-N_{120}$	7.50
$N_{120}-N_{160}$	0.67
N_{160}	— 5.66
$K_{40}-L_{160}$	2.1

cases. The effect of potassium treatments showed an increasing tendency but it did not produce a reliable crop surplus.

Studying the effect of the nitrogen treatments, it could be established for the average of the four potassium treatments that all the N treatments produced a significant crop difference compared to the N_{40} kg/ha treatment. There is no reliable difference between the other N treatments. Examining the effect of potassium treatment, it can be seen in the average of the four nitrogen treatments that only the K 120 kg/ha and 160 kg/ha treatments were significant compared to the 40 kg/ha K treatment. There is no reliable crop difference between the other potassium treatments.

In the course of evaluating the grain yield data, it could be established that the crop can be increased by approx. 15 q/ha by fertilization under experimental area conditions. The most favourable effect can be achieved with the application of 120 kg N/ha and 120 kg K_2O/ha. In the case of nitrogen the linear component of the effect is significant till the 120 kg/ha dose, beyond this the effect of the quadratic component, is also significant. With potassium the effect is significant till the 160 kg/ha dose, but only the linear component.

The nitrogen amount which ensures the optimal crop yield under the given experimental conditions was determined from the experimental data. This was 124 kg/N/ha. The confidence limits of this optimal dose of N with 90% probability were 88 and 152 kg N/ha.

It was possible to calculate the grain- yield surplus for 1 kg nutrient. The data referring to this are shown in Table 6. In the case of nitrogen, since both component effects were significant all N levels are given, in the case of potassium, since only the linear effect was significant, it was presented as the average of the four N levels. With respect to the N values, at N_{160} there were already negative values. In absolute numbers the maximal 19.65 yield surplus under dry conditions can be considered as a quite high value and the 13.32 grain-yield surplus at the 40—80 kg/ha application can be regarded as favourable. The grain-yield surplus for 1 kg potassium is 2.1 kg in the average of the four levels which means a rather weak fertilizer application.

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Prepared at the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest

B. LASZTITY

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METABOLIC CHANGES IN MUNGBEAN (PHASEOLUS AUREUS L.) SEEDS DURING GERMINATION

Different aspects of metabolism associated with germination have been the subject of study by many workers like STEWARD—STREET (1947), OOTA—FUJII—OSWA (1953), SIRCAR—DUTTARAY (1961), MAYER—POJLAKOFF-MAYBER (1963), INGLE—BEEVERS—HAGEMAN (1964) and MARCUS—FEELEY (1964). Metabolism of a dark germinated seed that has not been supplied with any external nutrition involves redistribution of reserve-food matters initially present in the endosperm or cotyledon with a portion being consumed in respiration. Germination leads to loss in dry weight and largely involves the association of embryo growth with respiration (TOOLE—HENDRICKS—BORTHWICK—TOOLE 1965). Previously, PAUL—MUKHERJI—SIRCAR (1970) worked on the activities of different hydrolyzing enzymes in mungbean seeds during germination. The object of the present work is to evaluate the daily loss of reserve materials from the cotyledons, the forms in which the nutritional requirements of the embryonic axis are fulfilled and to observe the accompanying respiratory changes.

Pure-line mungbean (*Phaseolus aureus* L. cv. NP 23) seeds were supplied by the Indian Agricultural Research Institute, New Delhi. Batches of weighed seeds after surface sterilization with 0.1 per cent mercuric chloride solution were washed several times with water. The washed seeds, after initial imbibition for 3 hours in a beaker, were spread over moist filter paper in Petri dishes and were allowed to germinate in a dark and humid atmosphere at a constant temperature of 30 °C in a germinator. Germinated seeds were taken out at intervals of 24 hours and were separated into axes and cotyledons. The experiment took five days. Seeds soaked in water for 3 hours were taken as initial or 0- hour samplings.

Weight determination: Fresh weight was taken by absorbing the surface water of the tissues with blotting papers. For dry weight, materials in definite quantities were kept at 60 °C in an oven for 4 days then the dried samples were weighed again.

Respiration: Respiration rate was measured at 30 °C in Warburg's apparatus following the manometric technique by UMBREIT—BURRIS—STAUFFER (1961).

Carbohydrate analysis: Fresh material was killed in 80 per cent ethanol and then extracted with the same solvent. Alcohol was removed by boiling the solution in a water bath. Direct reducing value was estimated by the copper reduction method of HARDING—DOWNS (1933) as modified by VAN der PLANK (1936). The total reducing value was estimated by determining the reducing value after hydrolysing with sulphuric acid. The sucrose content was represented by the difference between the total and direct reducing values. From the alcohol-insoluble residue starch was estimated colorimetrically according to the method of MCCREADY—GUGGOLZ—SILVIERA—OWENS (1950).

Nitrogen estimation: Nitrogen estimation was done in micro-Kjeldahl apparatus modified by Parnas and Wagner as described by PREGL (1930). The total nitrogen was determined from oven- dried material. Soluble nitrogen was estimated from an aqueous extract of fresh materials after trichloroacetic acid precipitation of proteins. Protein nitrogen was calculated from the difference between total and soluble nitrogen values.

Amino acids and amides: An 80 per cent ethanol extract of the fresh tissues was passed through a Dowex- 50×8 resin (H^+ form) column according to PLAISTED (1958). Elution of amino acids was made successively with 0.4 N NH_4OH, 80 per cent ethanol, 4 N NH_4OH and water. The combined elutes were concentrated in a vacuum and the residue dissolved in 1 to 2 ml of 10 per cent (v/v) isopropanol.

The detection and quantitative estimation of amino acids were made by the paper-chromatographic technique according to the method of THOMPSON—STEWARD (1951) and PORTER—MARGOLIS—SHARP (1957). In Whatman no. 1 filter paper resolution of the amino acid mixtures was made two-dimensionally by using phenol: water (4 : 1) and n-butanol: acetic acid : water (4 : 1 : 1) as solvents. 0.1 per cent ninhydrin solution was used as spraying reagent. Colour density was measured with a Klett—Summerson colorimeter using green filter (500—560 mμ).

Estimation of DNA, RNA and protein: Estimation of nucleic acids was made according to the method of SMILLIE—KROTKOV (1960). From 0.3 N KOH hydrolysate of sugar and lipid-free residue DNA was precipitated by 0.2 N perchloric acid in cold. DNA precipitate was hydrolyzed and measured at 600 mμ by diphenylamine reagent according to BURTON (1956). Supernatant RNA was passed through a Dowex- 1×8 (Cl^- from) column. Elution was made with HCL—NaCl mixture. Ribose content was measured by colour reaction with orcinol reagent at 660 mμ.

For protein determination, a portion of 0.3 N KOH hydrolysate was treated with 30 per cent TCA. The precipitate was dissolved in 0.1 N NaOH. From an aliquot of it protein was measured according to LOWRY —ROSEBROUGH—FARR—RANDALL (1951).

The results represented are the average values of four replicates.

The length, fresh and dry weights (Table 1). Epicotyl—hypocotyl axis increased about 24 times in length after 120 hours of germination. Both fresh and dry weights of the axis went on increasing. The cotyledons started to lose their dry weights after embryo growth began. Maximum depletion occurred during 72 to 96 hours.

Table 1

Length, fresh and dry weights of mungbean seeds during germination

Hours of germination	Length of axis (cm)	Fresh weight (mg/100 plant parts)		Dry weight (mg/100 plant parts)	
		Axis	Cotyledon	Axis	Cotyledon.
0	0.35	0.249	2.075	0.064	1.161
24	1.23	1.235	2.657	0.173	0.822
48	3.71	3.965	2.676	0.557	0.590
72	4.64	7.678	1.124	0.686	0.473
96	7.72	11.651	0.677	1.301	0.200
120	8.36	20.775	0.528	1.391	0.125

Respiration (Fig. 1). The respiration rate of the axis increased rapidly up to 96 hours and thereafter it slowed down. Oxygen consumption in the cotyledon proceeded at a slower rate and after 72 hours the rate declined.

Carbohydrate (Fig. 2). In the axis all the sugar fractions viz. total, reducing and sucrose increased with time while in the cotyledon depletion of these was noted. Reducing sugar content in the cotyledon was very small in comparison to sucrose. Rapid depletion of starch from cotyledon was noticed as soon as the seed was soaked with water. After 120 hours of germation the strarch content in the cotyledon was reduced to a minimum value. The axis

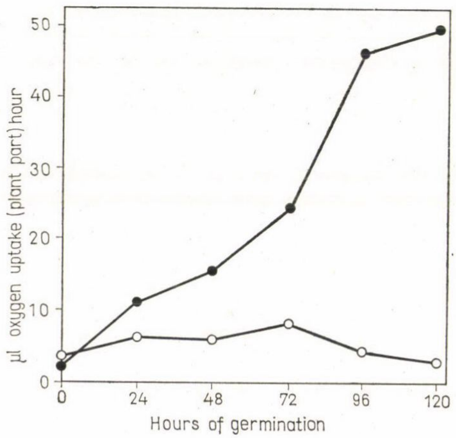

Fig. 1. Change in respiration rate [(μl oxygen uptake) plant part/hour] in mungbean seeds during germination. (●) Axis, (○) cotyledon

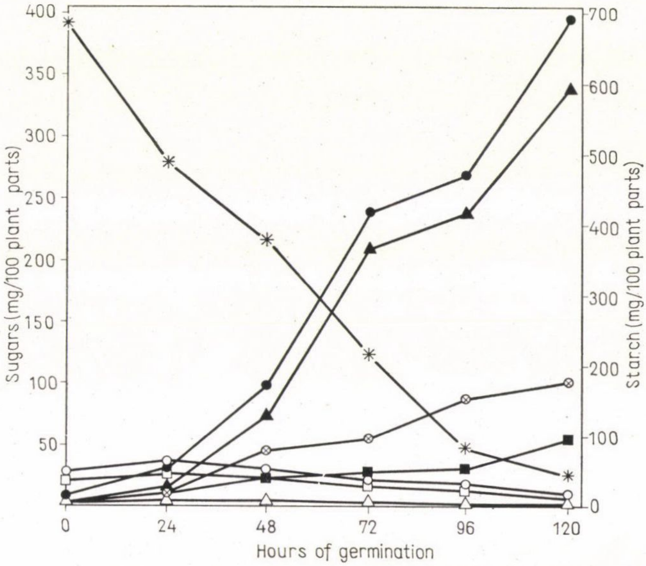

Fig. 2. Change in carbohydrate content (mg/100 plant parts) in mungbean seeds during germination. Total sugar—(●) Axis, (⊙) Cotyledon; Reducing sugar—(▲) Axis (△), Cotyledon; Sucrose—(■) Axis, (□) Cotyledon; Starch—(⊗) Axis, (✳) Cotyledon

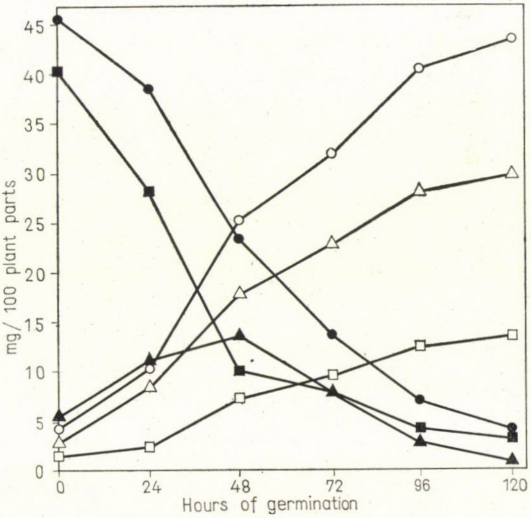

Fig. 3. Change in nitrogen content (mg/100 plant parts) during germination. Total nitrogen—(○) Axis, (●) Cotyledon; soluble nitrogen—(△) Axis, (▲) Cotyledon; Protein nitrogen—(□) Axis, (■) Cotyledon

tissue on the other hand showed starch formation at a slow rate from the beginning which increased considerably at the end of five days.

Nitrogen (Fig. 3). The total, soluble and protein nitrogen showed a remarkable rise in the axis during germination. Rise in soluble nitrogen was much higher than protein fraction. Both total and protein fractions of cotyledon fell rapidly with the initiation of germination,

Table 2

Amino acids and amide of mungbean seeds during germination
(mg/100 plant parts)

Amino acids	Parts	Hours					
		0	24	48	72	96	120
Aspartic acid	Axis	32.5	73.7	244.7	429.9	397.7	527.6
	Cotyledon	45.2	115.9	129.4	98.5	93.4	32.3
Glutamic acid	Axis	45.5	90.6	333.1	1292.6	1497.0	1446.1
	Cotyledon	71.0	194.4	207.3	63.0	—	—
Serine	Axis	34.5	108.2	476.6	—	—	—
	Cotyledon	42.7	120.7	120.2	46.0	—	—
Asparagine	Axis	—	—	—	620.8	766.1	781.8
	Cotyledon	25.5	48.2	57.5	31.4	30.4	27.7
Glycine	Axis	—	—	188.5	257.2	540.3	555.5
	Cotyledon	—	—	51.2	15.7	9.9	—
Threonine	Axis	22.9	130.7	449.0	590.4	—	—
	Cotyledon	73.6	77.0	168.3	—	—	—
Alanine	Axis	89.1	162.9	389.9	395.1	1532.0	1500.0
	Cotyledon	53.6	90.5	92.1	61.9	108.6	50.1
Arginine	Axis	—	158.5	503.8	1153.9	1293.5	—
	Cotyledon	59.3	99.2	188.1	96.9	48.6	—
Lysine	Axis	10.7	184.5	307.2	530.6	891.3	1514.5
	Cotyledon	—	—	—	—	8.5	34.5
Tyrosine	Axis	—	56.9	879.1	2364.2	2666.1	—
	Cotyledon	70.4	146.6	252.0	66.5	58.3	29.4
Tryptophane	Axis	38.1	60.4	312.7	746.8	798.4	752.6
	Cotyledon	27.6	73.0	92.5	36.6	18.3	13.0
Valine	Axis	—	94.9	452.0	980.7	1105.5	747.1
	Cotyledon	28 3	108.6	137.7	64.5	59.9	18.3
Proline	Axis	—	—	825.0	3450.0	2875.0	900.0
	Cotyledon	—	262.5	481.2	112.5	125.0	25.0

(—) absent.

while the soluble nitrogen level, initially representing smaller fraction of the total nitrogen, increased up to 48 hours and then declined.

Amino acids (Table 2). The number of amino acids in the axis at 0 hour was six which included aspartic acid, glutamic acid, serine, threonine, alanine and tryptophane. After24 hours four new acids— arginine, lysime, tyrosine and valine appeared and in the next 24 hours glycine and proline appeared. After 72 hours serine was replaced by asparagine. At the end of 120 hours nine amino acids were detected in the axis tissue. Initially the cotyledon contained ten amino acids. New amino acids to appear were proline and glycine. After five days of germination eight amino acids were detected in the cotyledon.

Aspartic acid. The concentration of aspartic acid went on increasing all through the experimental period in the axis, while in the cotyledon the peak rise was noted after 48 hours.

Glutamic acid. The formation of glutamic acid followed the same trend as aspartic acid, although the quantity of the former was much higher than that of the latter.

Serine. In the axis the level of serine showed a 14 fold rise up to 48 hours. In the cotyledon the peak rise was noted during the same period.

Asparagine. The maximum rise of the asparagine content was found during 48 hours in the cotyledon. However, in the axis it appeared after 72 hours of germination and increased for the next two days.

Glycine. Appearing in both types of tissues during 48 hours the glycine content increased in the axis and declined in the cotyledon.

Threonine. During its presence up to 72 hours in the axis and 48 hours in the cotyledon, the threonine concentration showed a significant rise.

Alanine. In the axis the alanine content increased 16 fold after 120 hours of germination, while in the cotyledon the rise was slow up to 96 hours and then declined.

Arginine. The most rapid rise of the arginine content occurred between 24 to 48 hours of germination. In the cotyledon it increased up to 48 hours and then declined.

Lysine. With little initial content, the axis showed 141 fold rise in lysine after five days of germination. However, it appeared during 96 hours in the cotyledon and increased about 4 times during the next 24 hours.

Tyrosine. As a result of germination the concentration of tyrosine increased remarkably in the axis. The cotyledon showed a maximum rise during 48 hours which was followed by its decline in the subsequent hours.

Tryptophane. The accumulation of tryptophane in the axis continued throughout the experimental period, while in the cotyledon the rise was noted up to 48 hours and then decreased.

Valine. A steady rise in the valine level of the axis was maintained up to 96 hours, while in the cotyledon it increased to a maximum after 48 hours and then declined.

Proline. In the axis there was a maximum rise in the proline concentration at 72 hours and the corresponding peak for the cotyledon was during 48 hours of germination.

Nucleic acids (Fig. 4). Both RNA and DNA increased steadily in the axis during germination. In case of RNA this increasing trend was maintained up to the end of the experimental period. The DNA concentration however, declined after 96 hours. The cotyledonary nucleic acid level decreased with the progress of germination.

Alkali-soluble protein (Fig. 4). Exhaustion of cotyledonary protein proceeded rapidly with the initiation of germination. In the axis the protein level increased up to 96 hours and then declined.

As there is no external supply of nutrition, it is obvious that the gain in the dry matter of the embryo is the consequence of the dry- weight depletion from the cotyledon which ultimately becomes exhausted. Rate of loss in dry weight from the cotyledon is maximum between 3 to 4 days of germination. In germinating barley seeds most of loss from the endosperm occurred between 2 to 8 days (Folkes—Willis—Yemm, 1952). After 5 days the dry weight which had accumulated in the axis was about 23 times the initial content at 0 hour. Beevers—Guernsey (1966) found 30 fold rise in axis dry weight of pea seeds after 14 days of germination.

The switch-over of the dormant embryo to the active phase of germination seems to be the cause of the rapid rise of respiration rate within 24 hours of imbibition. It is also interesting to record that the high rate of respiration in the axis between 72 to 96 hours (Fig. 1) commensurates with a maximum rate of dry-matter accumulation during this period (Table 1). This seems to be due to the release of energy for carrying on synthetic activities. After

72 hours the rate of oxygen consumption in the cotyledon falls, as the food matters are exhausted, This is in accord with James—James (1940) who found that the decline in endospermic respiration of barley was due to disappearance of starch.

The starch degraded rapidly from the cotyledon with the beginning of germination followed by the transport of soluble sugars to the axial tissues (Fig. 2). As both cotyledon and axis contained a little amount of the total sugar initially, the large increase of the same in the axis at the final phase of germination was the result of the breakdown of reserve starch. During the course of germination the sucrose fraction of the total sugar in the axis gradually declined with a corresponding large increase of reducing sugar suggesting the consumption of sucrose as respiratory substrate. This view is in accord with the observations of James (1940), Nada—Rafaat (1955). However, it is seen that out of the amount of starch- breakdown products translocated to the axis a little less than one fourth has been utilized to form starch at the end of 120 hours germination and the rest simply increased the total sugar level. In germinating mungbean seeds it has been reported that starch-breaking enzymes or α-amylases are quite active both in the cotyledon and in the axis from the start of imbibition (Paul—Mukherji—Sircar, 1970). Starch utilization in mungbean begins from the first days of germination.

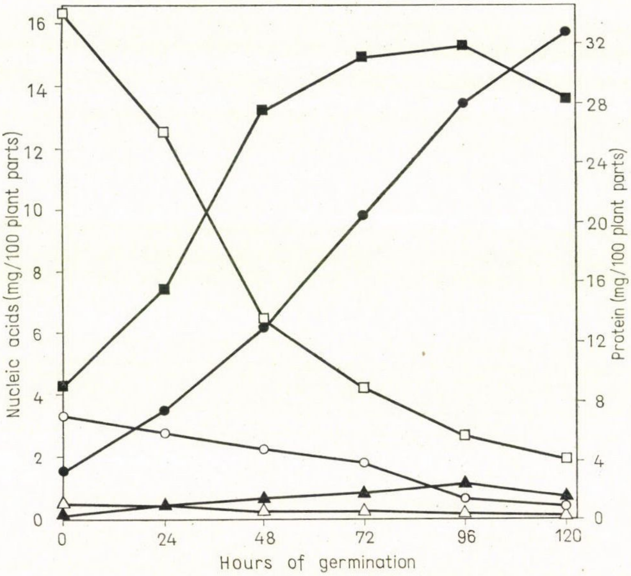

Fig. 4. Changes in RNA, DNA and alkali- soluble protein content (mg/100 plant parts) mungbean seeds during germination. RNA—(●) Axis, (○) Cotyledon; DNA—(▲) Axis, (△) Cotyledon; Protein– (■) Axis, (□) Cotyledon

Consumption of cotyledonary protein nitrogen proceeded at a much faster rate than soluble ones, suggesting protein hydrolysis with the initiation of germination. A substantial amount of resulting soluble nitrogen was transported to the axis and thereby increased its content. Protein synthesis began slowly within 24 hours of imbibition and after 5 days of germination nearly one third of the reserve protein initially present in 0 hour cotyledon could be recovered in the axis (Fig. 3). According to Marcus—Feeley (1964, 1965), the start of protein synthesis in an imbibed seed is due to polysome formation. The major bulk of soluble nitrogen which remained as such in the axis and could not get converted to protein was due to the inability of the dark germinated seedlings to transform it to protein (**Steward—Durzan**, 1965). Soluble nitrogen remained mostly in the form of amino acids and amides, as the number and concentration of them increased during germination. In rice seedling **Sircar—Duttaray** (1961) found that protein synthesis occurred after 72 hours of germination.

In view of the fact that some new amino acids appeared and few disappeared during germination, it is likely that a large-scale interconversion occurred among them (Table 2). For example, a fall in the proline concentration in the axis after 96 hours may have a reflection on the increase in glutamic acid and arginine content as these are metabolically interrelated (**Yemm—Folkes** 1958). Actually **Sivaramakrishnan—Sarma** (1956), by using C^{14}-glutamic acid, have found that glutamic acid could be converted to arginine and proline in green gram seeds. The appearance of glycine both in the cotyledon and the axis after 48 hours and its increasing concentration in the axis may be related to the disappearance of serine. Asparagine is present in the cotyledon throughout the experimental period. However, it appeared after 96 hours in the axis and its concentration remained much higher than that of cotyledonary asparagine. Accumulation of asparagine serves as the store of amide nitrogen from which amides released as ammonia may be utilized in the synthesis of new amino acids (**Street** 1966). In the cotyledon concentrations of most of the amino acids attain their peak after 48 hours of germination which gets a strong support by the similar peak rise for soluble nitrogen level during the same period (Fig. 3). Parallel to the rise in soluble nitrogen in the axis amino acid concentrations also increased in general.

Amount of RNA and DNA transported to the axis was much higher than the corresponding loss from cotyledon within 24 hours of germination which suggests synthesis of nucleic acids even from the earlier periods of imbibition (Fig. 4). A portion of soluble nitrogen may be utilized in the formation of nitrogenous bases for the building up of nucleic acids. With the growth of the axis, cell division and cell elongation proceed rapidly and thus nucleic acid concentration increases. **Ingle—Hageman** (1965) have noticed *de novo* synthesis of DNA and according to **Commoner** (1964) the nucleotides serving as energy source for synthetic processes are diverted into DNA molecules and result in the formation of new DNA during cell division. After 96 hours there is a rapid fall in RNA level of cotyledon which commensurates with the peak rise in RNase activity during the same period (**Paul—Mukherji—Sircar** 1970). Although it is difficult to comment regarding the nature of RNA, the findings of other workers (**Dure—Waters** 1965, **Mitra—Chakrabarty—Sircar** 1966) reveal that messenger-type RNA is formed during germination.

The ratio of alkali- soluble protein and RNA gradually increases in the axis during germination (Fig. 4). In a germinating seed it is difficult to predict the involvement of RNA in protein synthesis. However, in maturing beanseeds, protein synthesis is a linear function of the total RNA (**Oota** 1964). On the other hand, **Barber** (1962) found that the dcrease of RNA from *Vicia* cotyledon was associated with the synthesis of albumin suggesting that the presence of RNA is not necessary for the building up of protein.

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Prepared at the Department of Botany, Calcutta University, Calcutta-19 .

A. K. **Paul**, S. **Mukherji**, S. M. **Sircar**

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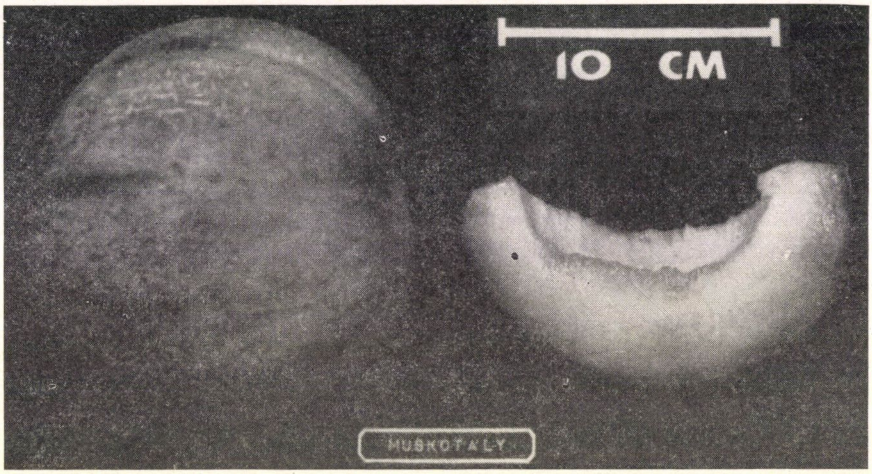

MUSKOTÁLY

Taxonomic place: Cucumis melo L. ssp. *melo* MSF.

Origin: produced from the variety "Zöldhúsú Ananász" by individual selection and evaluation of progeny.

State qualification: provisionally certified variety 1958, state-certified variety 1964.

Beginning of breeding: 1950, Makó.

Breeder: János Bruder and Ferenc Szalay, Makó.

General characterization: a very tasty variety with thin rind and soft flesh (difficult to transport, therefore only suitable for local consumption), medium productivity and long vegetative period.

Morphological description:

Root system: strong, effuse.

Shoot system; procumbent, vigorous.

Foliage: large, medium green, pentagonal, slightly lobed leaves with a petiole shorter than the leaf blade.

Flowers: with brimstone-coloured corolla.

Fruit: spherical, slightly ribbed; thin rind, green when ripening and straw-yellow when ripe, yellowish green between the ribs. Surface slightly corky, flesh sweet, spicy, light green and very soft when ripe (cannot stand transportation). Fruit cavity medium large. Average fruit weight 0.85 kg (Tuza 1968). As to its taste, it belongs to the best varieties.

Seed: cream-coloured: thousand-grain-weight 25 g.

Biological character:

Development: slow, protracted.

Vegetation period: 113—140 days from emergence to first picking: picked over 50 days: a medium late variety.

Resistance to disease: in rainy years susceptible to *Colletotrichum lagenarium*.

Farm-technology requirements:

Seeding: in hot bed in the first half of April, transplantation in the first half of May.

Soil requirement: warm, freshly manured.

Productivity: medium fruit yield ranging between 43 and 120 q/ha: the bulk of the yield is picked in the fourth to sixth week of the harvesting period.

Region of cultivation: grown only in private gardens in the southern part of Hungary: owing to its bad transportability, only sold on local markets (Tuza 1968).

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Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen

Gy. Mándy

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FORUM

CONTRIBUTIONS TO THE PAPER OF GY. MÁNDY: EXPERIMENTS WITH THE "HORSE-RACE METHOD" OR OTHERWISE? PUBLISHED IN THIS PERIODICAL, 23, (1—2)

IS THERE ANY RELATIONSHIP BETWEEN THE RESPONSE OF VARIETIES TO SOWING TIME AND THE AMOUNT OF YIELD?

A generally used method of evaluating the economic efficiency of plant varieties is to study their relative productivity, quality of yield, resistance to diseases and pests and other qualitative features under various soil and climatic conditions usually over three years in comparative trials. Varieties considered best to be grown in a given region are selected on the basis of observations and measuring made for several years at different sites. Recently the variety trials have been improved by testing the varieties at various levels of nutrient supply in order to get information on their nutrient response, to find out how they are able to make use of abundant nutrient supplies, and how the different rates of fertilization modify the qualitative features of varieties (quantity and quality of yield, lodging resistance, etc.).

György Mándy wishes to improve this generally applied evaluation method of plant varieties; he suggests performing sowing time experiments with the varieties to be tested, instead of repeating the experiments for years, or testing the varieties in different regions.

We fully agree with the opinion that the yield of a variety considerably depends on the time of sowing. This has been proved by a great many experiments. Further, varieties are known to show different degrees of response to sowing time, and to deviations from the optimum time of sowing. Perhaps the largest number of experiments have been performed with wheat, and for example, according to our experiments too, the winter-wheat variety Fertődi 293 excelled in tolerating late sowing better than many other varieties, that is, when sown in November or December its yield was reduced to a lower extent compared to the most favourable mid-October sowing than the yield of the winter variety Besostaya 1. This property is highly valuable in practical wheat production, and the more so when the farm works under extensive conditions, because in this case a considerable proportion of the wheat will often be sown late. But it occurs even under intensive conditions that a plot is only sown in November. This property cannot, however, be the primary condition at the growing of a variety, since the farms sow the overwhelming part of the wheat in due time, and it is those varieties that give the highest yields under such conditions that deserve attention in the first place. On the contrary — keeping to the above example — the variety Besostaya 1 ought to be replaced by Fertődi 293.

The other question is whether a several years testing of varieties can be safely replaced by the experience of periodical sowing. To this we must unanimously answer no. Again using wheat as an example, it is in vain carrying out periodical sowing since, if a mild winter follows, it will not be possible to make sure about the winter hardiness of the variety. But the effects of changing weather conditions in spring and early summer cannot be assessed in a year either. Alas, the judgement of varieties can thus hardly be accelerated by periodical sowing. Similarly, the examination of regional effects cannot be replaced in this way either.

The situation is similar with plants sown in the spring. With a part of them the problem is made even more difficult by the photoperiodical sensitivity as a consequence of which

sufficient results cannot be obtained with late sowing even under favourable temperature and precipitation conditions.

Of course, all this does not mean that it would not be useful to carry on detailed agrotechnical experiments — including sowing-time treatments — with the varieties proved best in the variety trials and recommended for general production, in order to be able to give detailed information to the practice of growing on the most appropriate cultivation system of the variety.

The other suggestion of the paper cannot be analysed in detail on the basis of the very brief summarization. We only wish to note that the "individual amplitude" depends — among others — on the genotype of the variety, the quality of the seed-bed, the method of sowing, in the case of cereals on the tendency to tillering and also on the factors influencing the extent of tillering. The "lowest amplitude value of the dough stage" can be found in uniformly emerged, moderately tillered wheat, that is why it "agrees with the highest value of grain number and spikelet number per ear".

G. LÁNG

University of Agricultural Sciences,
Department of Plant Growing,
8360 Keszthely
Deák F. u. 16.

IS FRACTIONAL SOWING NOT A "HORSE-RACE" WITH DIFFERENT STARTING TIMES?

A basic principle — probably the most important one — of experimentation is that with the exception of the factor to be studied all the other circumstances should as far as possible be identical. This principle need not even be explained, since, if in an experiment besides the factor to be examined another factor were also changed, the joint effect, or in most cases the interaction of the two factors would prevent us from finding out how much the result obtained depended on one, and how much on the other factor. An application of this principle is the case when in the variety trials variety is the only changing factor, and the other factors, including the sowing time, are as far as possible identical in all treatments. Furthermore, in variety trials serving practical purposes, sowing is carried out at a time optimum for the plant species concerned, in order to make the inherited productivity of the varieties come into full display.

In our agricultural literature, however, it was pointed out very early that such a perfect identity of factors other than the one examined may have a disturbing effect, or even give misleading results. As early as in 1887, Sándor Cserháti e.g. wrote that — since the space requirements of the maize varieties are highly varying — without a knowledge of the optimum spacing of the varieties "no reliable comparative yield trials can be carried out" (CSERHÁTI 1887). Namely, in an experiment laid out with identical plant numbers it may occur that a variety attains the highest yield because the spacing applied in the experiment is optimum for it rather than because its inherited productivity is the best, while other varieties would give much higher yields when spaced closer.

In his paper György Mándy places the time of sowing among the factors whose strict identity changes the result of the variety trial, because in an experiment with the same sowing time applied "the maximum output of varieties cannot be concluded on". He gives the reason in: "each variety (stand) has special ecological requirements". This statement is theoretically correct, but in the practice one can hardly imagine, e.g. a winter wheat variety that would not need the absence of permanent drought or severe autumn frosts after sowing. Thus, in many cases the fact is that every variety gives a favourable response to conditions promoting its development, the only difference is that some varieties suffer more from the lack of such conditions than others. To put it in a different way: varieties with higher demands are more reactive to the optimum conditions. This is clearly shown e.g. by the data of the wheat experiments at Keszthely (KOVÁCS 1970), where all four winter wheat varieties studied in 1961/62 gave the highest yields when sown at a time ensuring the best conditions (6 October). However, they showed great differences in tolerating unfavourable, late sowing; the yield of the more resistant Hungarian variety Fertődi 293 was only reduced by 18 per cent, while in the foreign varieties the yield decrease was 29, 37 or even 44 per cent!

Since according to the above, different sowing times provide different intensities of ecological conditions for the varieties, it is a highly valuable idea to replace experiments carried on over successive years by variety trials sown at different times within the same year — even if such a shift of the sowing time does not produce quite the same differences as found between the weather conditions of different crop years. In such a way we can satisfy the requirement that the breeder "has to create extreme conditions . . . to find out what the behaviour of the variety concerned in such extreme years is" (VILLAX 1944).

Some authors called variety trials variety competitions as early as in 1950. The unusual term "horse-race method" however, is quite new. But if, with a view to determining the optimum sowing time for each variety, we sow them at different times of the same year, we do not eliminate the "horse-race method", on the contrary, we organize more "horse-races" with different times of starting. Namely, if we tried to sow the varieties at different times considered optimum for them in a single experiment, we might easily obtain extremely misleading results, the more so as we cannot — unfortunately — foresee the subsequent weather.

It is a correct statement in Mándy's paper that in a variety trial carried on over several years it frequently occurs that different varieties are found the best in the successive years. This is the very reason why the experiments should be conducted over a number of years; in this way we can establish the true value of the variety for the practice, since the yield "must be the highest possible not only in a favourable year but on a many years average" (BERZSENYI-JANOSITS 1956). Another statement of Mándy's paper, namely, that "the cause of these yearly differences is not searched for" cannot be generalized. To mention some examples to testify the opposite of this statement: variety trials with winter rape at Sőreg showed in 1939 Lembke, while in 1940 Eszterházai to be the highest yielding variety. The reason for the contradiction is given by the text as follows: "The later 'Lembke' variety which had higher cultivation requirements was only able to give a larger yield than 'Eszterházai' in the most intensive experiment of the favourable year of 1938/39" (BERZSENYI-JANOSITS 1941). In a similar way, the text presenting the results of the winter wheat variety trials describes in detail "how the different weather conditions of the three years influenced the productivity of the varieties" (PAPP 1953).

Mándy's statement that unfavourable conditions may increase the individual amplitudes within a plant stand to a great extent, is very interesting and remarkable. It is known that in an unfavourable crop year soil differences left unobserved in a favourable year can be strongly felt and may have a disturbing effect in the experiments. In the same way, the stress effect of an unfavourable sowing time may underline such minor differences either in the micro-environment or the hereditary character of the plant as could not be observed without such a stress effect, and so the individual amplitudes will be larger in the plant stand.

The stress effect of the unfavourable sowing time, however, can be directly measured by the yield; although the individual amplitudes are in close correlation with this stress effect, it would be a mistake to trace back the individual amplitude to an incorrectly chosen sowing time, since such a conclusion would show, e.g. 8 November to be the optimum sowing time of winter wheat (see Fig. 4. of the paper), which would be a palpable error.

Finally, another error has to be pointed out in Mándy's paper. He writes: "in the fractional sowing experiment sunflower proved to be more responsive to temperature than to precipitation. Still, according to the plant growing manuals sunflower has high "water requirements". On the contrary, all Hungarian plant growing manuals (GRÁBNER 1935, BITTERA 1930, VILLAX 1948, etc.) emphasize the high temperature requirements of sunflower and mention its drought tolerance. Even Láng (another book of whom is referred to by Mándy) wrote (LÁNG 1954): "Sunflower prefers higher temperatures . . . It tolerates drought very well". According to Kurnik — in the chapter "Climate and soil requirement" of the book referred to (KURNIK 1970) — "it tolerates dry weather, and even drought well". The term "high water requirements" cited in the paper and appearing contradictory to the text of the previous page is only found in the chapter on soil preparation, perhaps to underline how important a possible preservation of soil moisture during the soil preparation is. It would thus be a mistake to think that the fractional sowing experiment has rectified an erroneous statement of the Hungarian plant growing manuals.

L. BERZSENYI-JANOSITS

1124 Budapest
Tornalja u. 1.

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IS THE METEOROLOGICAL REACTION OF VARIETIES IDENTICAL WITH THE EFFECTS OF ECOLOGICAL CONDITIONS?

Considering that the weather conditions of Hungary more or less vary from year to year, and the climates of the different regions show nearly the same differences in the majority of the years, I fully agree with Professor Mándy's argumentation. The "horse-race" method of determining the properties of varieties must be considered unsuitable for establishing general rules valid for the variety concerned and for the whole country under any weather condition, as regards either the optimum sowing time, or any important phenological phenomenon.

Further, I must agree with Professor Mándy in his view about this phenomenon being caused by the so-called "ecological conditions", besides the genotypic properties. However, our opinions do not agree concerning the cause of the differences, inasmuch as I find it in most cases decidedly in the meteorological and climatic conditions alone. Namely, in the case of experiments carried out at a given site, the composition of the soil, the exposure as well as the cultural practices must be regarded as perfectly identical, and so must the living environment (man, animal and plant), therefore the effect of the environment manifests itself exclusively in the meteorological and climatic differences. No matter how carefully Mándy tries to designate this effect by the general term "ecological", or call it — similarly not quite exactly — the effect of the "crop year", it means singularily and exclusively the meteorological reaction of the variety.

The meteorological and climatic material and energy turnover may vary to a great extent from year to year. In successive years (e.g. 1966 and 1967), at the same place, irradiation and eradiation, air movement, temperature and precipitation are different before sowing, at the time of sowing and after, even if we consider the same date of the same season (e.g. autumn or spring). Winter wheat sown on 7th October is exposed to different meteorological effects almost every year, that is why the yield results of the same variety grown at the same site are different in successive years. On the other hand, winter wheats sown on 7th October are superior both in yield and quality to those sown in September or later in October. And the individual varieties show different results according to the meterorological response of the variety. The optimum time of sowing could be fixed even more exactly if fractional sowing were carried out with one- or two days- rather than a whole-week intervals. This is well demonstrated by Koltay's mentioned sowing-time experiment with two winter-wheat varieties over 3 years each.

When comparing the data with the weather conditions of the years concerned, we find numerical correlations of orientation character between the meteorological elements (or even element groups) and the results of the varieties examined. Dénes Berényi was the first to apply such correlations in Hungary. Today, in the age of computers, when regularities hidden in great masses of data can be established with a relative easiness, such a work would be very fruitful. It is obvious that in this case local meteorological observations would be indispensable, and three years would not be enough for an experiment series to provide reliable results that can be safely used in practice.

The reason why I regard the weather as one of the most important ecological factors is that this factor depends the least on man who is able to control the growing area and all the cultural practices at will, but is compelled to adapt his production activity to the meterological and climatic conditions.

This undeniable fact confirms my opinion that no variety should be grown at the same spacing all over the country, since different climatic conditions would justify different spacing. In the same way, it is decided by local meteorological conditions whether a variety should be sown early or late in a given region. That is why the results of Koltay's experiment cannot be regarded as of general validity for the whole territory of Hungary, although they are highly appreciated, all the more so, because they agree with the results of an earlier research work (András Kováts: A vetésidő, az elővetemény és a trágyázás hatása az őszi búza termelésére (Effects of sowing time, forecrop and fertilization on the production of winter wheat); Doctor's dissertation, Gödöllő, 1960. It is certain that in some regions early, while in other regions late sowing gives the best results.

I agree with Mándy's opinion that varieties sown at an optimum time will show the best quality and highest yield results, since influences exercised at the time of sowing are decisive and remain effective even at the time of harvest, except when some great disaster befalls the stand during the vegetation period (flood, internal water, drought or frost).

All in all, I can say that I agree with all statements in Mándy's paper, only instead of the general term "ecological conditions" I would mention in the first place the weather and climate as decisive factors causing the differences. Therefore, in determining the optimum sowing time of a variety, the "horse-race" method should be replaced by a careful consideration of the meteorological and climatic conditions.

† N. Bacsó
1122 Budapest,
Városmajor u. 1/b.

WHAT IS THE EFFECT OF THE DAY-LENGTH DIFFERENCE ON THE DEVELOPMENT RATE?

The major criticism on Dr Mándy's paper is that the physiological basis of the differences observed with different planting times is not fully enough considered. One of the probable variables to be encountered with such plantings is not even mentioned, namely the day-length difference. All plants are sensitive to photoperiod to some degree, and most certainly the variations reported by the author in yields, etc. with diverse planting dates are in part to be explained by differences in development rate occasioned by photoperiod. This is not to say of course that varied conditions of weather may not be encountered by populations planted at different times in the same year, which is the explanation proposed by the author.

My recommendation would be for Dr Mándy to reconsider the argument, taking into consideration the likely photoperiodic effect on development rate.

J. Heslop-Harrison

Royal Botanic Gardens
Kew, Richmond, Surrey, England

WHY IS A SINGLE EXPERIMENTAL DESIGN OR PROCEDURE NOT ADEQUATE IN THE DIFFERENT STAGES OF VARIETAL EVOLUTION?

Prof. Gy. Mándy has rightfully raised some questions relative to field experimentation, particularly the procedure he has designated as the "horse-race" method. Field experimentation is conducted to provide answers to certain specific problems. As varietal evaluation normally procedes sequentially, a design or procedure suitable for one stage may by quite inefficient or inappropriate for a different stage.

The geneticist-breeder faces the problem of evaluating relative field performance of large numbers of items under a meaningful set of differing environments, usually provided by different locations within a given season and repetitions over seasons. Other possible variables such as planting date, planting densities, etc., are held constant. The experienced inverstigator knows, as Gy. Mándy has pointed out, that no single entry will consistently rank first. Normally, however, the objective is not to find the single best variety but rather to identify a group of varieties having possible potential for extensive commercial use. Having identified such an elite sample other experimental procedures or statistical analytical approaches are indicate.

Given the data in Table 1, several types of questions may be properly asked, each question possibly requiring a different experimental approach. One very practical question of importance to the farmer would be: among this group of varieties which will produce the highest average yields? The data given are not adequate to answer this question but they do indicate that the varieties seem to fall into two groups: one having a mean of approximately 38 and the second with a mean of approximately 33. In view of the apparent high genotype—environment interaction there may be some question as to the meaning or practical significance of these gross differences. Knowledge of the material and of the seasonal conditions might suggest some explanation for the differential seasonal response. Practical considerations suggest that the "best" variety will be the one having a high average performance and the greatest stability of yield under the range of environments under which it will normally be grown.

The data in Tables 2 and 3 and the several figures emphasize the fact that each variety performs best under some specified cultural regime (planting date, population density, etc.). Detailed data required to establish such specifications cannot be accumulated for all products from a breeding program; such differences can only be explored with a selected sample of superior material. In such studies, experimental comparisons must be undertaken in all of the recognized ecological zones in which the production of the crop is of importance. Variety A may be best in Zone I while Variety C may be best in Zone II; "best" meaning the highest average yield and greatest stability of yield under the normally prevailing conditions of culture.

Gy. Mándy is correct in pointing out the limitation of a system he has designated the "horse-race" method. He would have been equally correct in emphasizing that no other single system of testing is adequate. The proper experimental desing and procedures appropriate to a given situation are determined by the questions for which answers are being sought.

G. F. Sprague

Department of Agronomy,
University of Illinois,
Urbana, Illinois 61801,
USA

SHOULD THE DETAILED CROP- PHYSIOLOGY EXPERIMENT REPLACE ALL OTHER TYPES OF EXPERIMENT?

The question of method of field experimentation raised by Dr. Gy. Mándy opens up the whole subject of genotype x environment interactions and their interpretation. These interactions are very real and of the utmost importance. However, I think it is necessary to put field experimentation into perspective and to consider what aims an experimenter may have in mind.

The experimenter may be interested in, (a) investigating for fairly immediate information, for example;

1. the potential yield of a variety in a given situation,
2. the optimum cultural practices for a variety,
3. the yield and adaptability of a new variety, with a view to recommendation for commercial use; or (b) the longer- term research experiment to investigate the factors which influence yield, and so lead to a better understanding of crop growth and possibly the formation of hypotheses or the development of techniques to bring about further improvements.

The basis of all experiments is the use of proper statistical layout and replication so that it may be stated with reasonable confidence that differences found were not due to mere chance. To do this invalidates to some degree the "horse-race method" criticism levelled by Dr. Mándy. It is also necessary to evaluate the factors which contribute to the G×E interactions and to give appropriate weight to them in the execution of the experimental programme. Three ovious factors are time of sowing, fertility and season. Only the first of these has Dr. Mándy advocated studying in detail although for any commonly grown crops the approximate optimum dates are usually already known and adhered to in practice. Doubtless small interactions of variety x sowing date exist but these would be greatly overshadowed by place - to - place and even more by year - to - year variations.

From a plant breeder's viewpoint the problem is to assess and compare many hundreds of segregates. This is basically a problem of logistics and the large number to be tested must be reduced to something more manageable in "once only" type experiments, with replication but without other variables, in the hope of "creaming off" the best few. The inclusion of other variables, such as a range of sowing dates, would necessitate a drastic reduction in the number of genotypes that could be tested and thereby reduce the chances of fully exploiting the genetic variation.

Once the better lines have been selected, these are then usually tested in a series of experiments at a number of sites and over several seasons to get a measure of their general yielding ability and adaptability. For this purpose it is usually considered that trials in different years are better than the same number of trials, even at different sowing dates, in a single year. This is because, in most cropping areas, differences in climate between seasons are usually larger than any differences that can be experienced, even with manipulation, in a single season. The best that can be done is to look at performance in a number of locations and in a number of years and to choose the variety which appears to perform well most consistently. A technique of considerable value in the assessment of adaptation and genotype x environment interactions from a series of experiments has been described by FINLAY—WILKINSON (1963).

To turn to a more specific discussion of the value of assessing material in an experiment which, although carried out in one season, includes a whole sequence of sowing dates, Dr. Mándy's Table 1, which shows the fluctuating performance of different varieties of peas over three seasons, illustrates the point he makes as to the dangers of basing assessment on one trial only, assuming statistical analysis has indicated that these varieties were in fact significantly different. He has not, however, included any Standard Errors. The mean of the three seasons suggests that Perfection Dark Skinned is almost certainly the most reliable variety and, if S.E's had been presented it would probably have been shown that this variety was not statsticelly worse than the highest yielder in any of the three seasons. No experimenter would throw out this variety on the results of any of the individual trials.

The point is made that "no conclusion can be drawn concerning the maximum yields of the varieties" if the optimum sowing date does not happen to have been sampled. However, the potential yield may not be measured at all in some seasons even if a range of sowing dates has been considered (e.g. Table 2, 1965 yields). The variable "tested" in a sequential sowing date experiment is the change in climate within the one season. Other variables which might be equally important and which would have occurred if a number of sites had been used such as: intrinsic fertility level of the soil, its pH and moisture-holding capacity, and the incidence and severity of disease, have not been experienced. It is the interactions of all these variables (and many others) that determine yield. Even then there are good and bad years, what are these due to? The really interesting comparison in Table 2 is between the yields of Bezostaya 1 in 1965 and 1966! Large differences can occur between seasons and the climatological and ecological differences between these are also worthy of study.

Crop- physiology studies, which relate crop growth and yield to environmetal and climatological influences, explain how crop yields have developed in retrospect provided all the necessary variables have been measured. These studies greatly increase our knowledge of crop behaviour but cannot predict results in advance. Simple correlations with yield can be grossly misleading, and where crop physiology studies are undertaken, greater elucidation is achieved if growth is broken down into sequential stages and yield into its component parts. A difficult problem in crop-physiology studies is to decide which variables and characters to measure. Mainly correlated evidence is accumulated and the danger of attributing results to factors which only have a partial or indirect effect, must be emphasized.

The depth of detail to which the various variables are to be measured is a further problem. The finding in one experiment discussed by Dr. Mándy (Fig.2) that "the sunflower is more sensitive to temperature than to precipitation" is a sweeping statement. Growth, in particular height, tends to be correlated with temperature, therefore it is no surprise that this correlation was found. However, it does not necessarily, as implied, nullify the statement that sunflowers have a "high water requirement". Good yields will not be produced where there is inadequate water, but what level is adequate? Crop water requirement can be satisfied by a soil profile with a high water-holding capacity, fully charged at the time of sowing and followed by well-distributed (in time) although sparse rainfall. Total rainfall, without regard to the size of individual stroms and their distribution, is a statistic of limited value.

Dr. Mándy is right to point out that the study of interactions with environment deserves more attention. Detailed crop-physiology experiments provide evidence to guide future thought and suggest lines of improvement. However, this type of experiment must not replace all others; there is room for a whole range of experimentation, some to service the more immediate needs. Whereas a "once only" experiment has its admitted disadvantages, it remains a fact that for each farmer his crop is a "once only" affair, without replication, and he requires the most relevant advice to ensure him the best chances of success.

D. A. Lawes

University College of Wales,
Welsh Plant Breeding Station
Plas Gogerddan,
near Aberystwyth,
Great Britain

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WHY IS THE OFTEN ONLY ACCIDENTAL SELECTION OF A SINGLE SOWING DATE INSUFFICIENT FOR THE ECOLOGICAL JUDGEMENT OF THE USEFUL AGRICULTURAL PLANTS?

The work of colleague Mándy has a high scientific value in the field of agricultural experimentation activity, for which I can only congratulate him. Naturally, I recommend that this work should be published unshortened, in full. The basic concept of the work is that Mándy rightfully points to the fact that exceedingly valuable insights can be obtained into the ecology, phenology, quality evolution and further physiological properties of useful agricultural plants, by arranging their sowing systematically at equidistant sowing dates. In this way the entire ecological spectrum of the respective species, race or variety is opened for the researcher. He rightly points to the fact that the often only accidental selection of a single sowing date is totally insufficient for an ecological judgement of the useful agricultural plants and that the practised way of laying-out experiments can very easily lead to aggravating, erroneous conclusions.

As I have already stated I can most warmly recommend the publication in an unabbreviated form and wish profoundly that this work should be made known not only to the Hungarian agrarian scientists, but to other agrarian scientists of the world, as well, because it contains a highly original idea which will promote the agrarian sciences to a very great extent.

J. Barner

Arbeitsgruppe Umwelftforschung und
Landespflege der Universität,
78 *Freiburg* i. Br.

WHY IS THE "HORSE-RACE" TYPE METHOD NECESSARY?

Gy. Mándy's paper "Experiments with the 'horse-race method' or otherwise?" published in Acta Agronomica, No. 23 1—2, 1973, is valuable in emphasizing one aspect of agronomical experimentation which is being increasingly considered by research workers.

Mándy, in illustrating his theories with numerous examples, quoted from his own and from other research articles, points out that the most commonly used method for conducting variety trials in the field is the one in which all the varieties to be compared are placed in identical conditions, like horses beginning a race, and that this can lead to misleading results.

With this type of method, in fact, if a variety is placed in conditions which satisfy its particular requirements, it will give better results and surpass other cultivars which would have given the best results, had they been grown in conditions favourable to their development.

In referring to experimental results, Mándy studies the problem concerning sowing dates. He observes that, when just one sowing date is used for the variety trials, besides being unable to evaluate the maximum possible yields for the varieties, serious difficulties arise in judging which variety is best, because, depending on the date chosen, each variety, in fact, will develop in more or less favourable conditions and therefore the best results will be obtained from one rather than from the other.

Mándy, therefore, suggests always conducting variety trials with various sowing dates in order to create an "ecological series" with different climatic trends.

He also suggests measuring the differences in rapidity of development observed for the plants within a stand, i.e. early or late; assuming that these differences are the crop's reaction to the environment, when the environment is unfavourable to the plant's development, these differences between the plants will be greater.

Once the amplitude of these differences is known, it can be used for the choice of the best sowing date for each variety.

Concluding, Mándy expressed the hope that the "horse-race method" would be eliminated and that in the future variety, fertilizer etc. trials would be carried out as ecological experiments.

It should, however, be pointed out that the horse-race method generally used for variety trials and for most agronomical trials, besides the negative or uncertain aspects emphasized by Mándy, also has various other aspects.

In most cases, the trials are not only carried out with the same sowing date, but also under identical soil fertility, fertilizer, plant density and available water-level conditions without considering the various possible requirements of the varieties, or in general, of the treatments being compared. For example, in a variety trial on wheat, where some of the varieties are not very resistant to lodging, while others are resistant, it is obvious that at a low nitrogen rate the former type will benefit, whereas at a high rate the latter would benefit. Thus the yield levels would differ in the two cases. Likewise, in variety trials, for example, on beets, tomatoes, corn or sunflowers, the plant density adopted could influence the yield results. This would depend on a lower or higher density, with regard to beet types which tend to give a high root weight or those which furnish high sugar content; with tomato cultivars, on the tall indeterminate type or the small, self-pruning type; with corn and sunflower varieties or hybrids, on early or late growth cycle etc.

In fertilizer experiments, for example, at different N rates, the effects of the plant density, or solutions and the frequency of irrigation may be observed and may have so much influence on the results that the higher or lower N fertilizer would appear to be effective.

The trial results are often affected by the locality where the trial is carried out, but in this case, if the locality chosen is typical of that area, the problem is much less serious because the experimental data can at least be considered as approximately valid for that zone, even if they cannot be generalized.

Often the various factors which characterize the trial conditions (climatic trend, physico-chemical characteristics of the soil, sowing and harvesting date, fertilization, irrigation, plant density, cultural treatments, etc.) interact and influence the yield results both in a uniform manner for all the treatments compared (and generally this does not alter the trial value), and in different ways depending on the treatments by altering the mutual relationships (and this can cause misleading results).

Therefore the "horse-race method" presents various difficulties and negative aspects for comparisons. What remedies are possible?

The most obvious remedy would certainly be that of conducting the trials by placing each variety or, in general, each treatment compared, in conditions which can be considered optimal in relation to all the factors that can be controlled by man and are capable of affecting yield results. This is almost never possible because the particular requirements of the compared varieties are often unknown (i.e., trials where recently constituted varieties are used) or generally because one does not know which conditions will permit a given cultural technique to express the best results, and because often the conditions which are considered to be optimal for a certain variety or a certain trial treatment are not really optimal in the trial conditions.

It would, therefore, be necessary to carry out complex trials in which the comparison is made at different levels of all or at least some of the principal factors which could influence yield. This way, each variety or each treatment compared would be able to find the situation which gives it the opportunity of expressing its best yield capacity within the various conditions, however, the combination possibilities would be so great that the trial would not be feasible.

The "horse-race" type method, where all the treatments are on the same level, today seems the only method capable on a first approximation of supplying a certain amount of indicative information at a feasible cost and obviously requires another series of experiments in order to eliminate the defects. It would be best, however, to repeat the trials in time and space, so that the treatments are compared within the widest possible range of conditions. The results obtained should only be considered valid in those conditions and their validity should only be generalized with a certain amount of prudence. It is clear that even with this trial method there are serious drawbacks because, for example, valuable material is lost by discarding new varieties which, under different conditions, would have supplied better results; whereas

others are cultivated and do not give satisfactory results. However, if these indicative trials are not carried out initially, it will not be possible to conduct further more complex trials, where the varieties of treatments, judged to be the best, can be compared at different levels of the principal yield-determining factors.

G. Venturi

Istituto di Agronomia Generale
Facoltà di Agraria
Università di Bologna, Bologna

CAN SUCCESSIVE SOWING REPLACE A SERIES OF EXPERIMENTS REPEATED IN SEVERAL YEARS?

The contribution is based on some new ideas with the aim of improving the experimental field techniques, especially to get more information in the shortest possible time. Although such an endeavour has to be praised, some conclusions are unacceptable in the form the author presents them. They are the following:

The statement, that more than one variety forms a well- designed experiment, is in some way superfluous. If there is not more than one variety or treatment, then there is no experiment. The statement that only one variant will give the best results is hardly accurate. Very often several variants show no significant difference, or no difference at all. The range alterationof varieties in trials repeated in several years is the result of an interaction, which has recently been studied very thoroughly by many authors using various methods, e.g. ecovalence by Wricke, Comstock-Moll model of analysis of variance, performance regression of varieties on mean performance of all varieties in years and locations, etc. by Finlay and Wilkinson, Eberhardt and Russell, Weiling and others.

I don't think that we can state on the basis of this one experiment that the sunflower is more sensitive to temperature than to precipitation. These relations seem to be less simple than is suggested here. Temperature and precipitation cannot be regarded separately, as their influence is highly correlated. What is decisive is not the amount of precipitation, but its distribution over the vegetative period. In this way I would explain also the discrepancies found in the experiment with wheat (Fig. 4).

Neither can I agree that successive sowing can replace a series of experiments repeated in several years. This would mean neglecting the effects of day length and furthermore the complex of meteorological factors, changing and forming the characteristic periods of a year.

The ecological effect can surely be demonstrated by plant development, but it is unwise to confuse development with growth rapidity. Growth and development very often do not correspond at all. In poor conditions growth can be limited, but development accelerated. On the other hand, the interval between the "quickest and slowest" plant in a stand is more often caused by genetical homogeneity of the individuals than by other causes. Of course, poorer conditions may lead to higher variability, which, on the other hand, would depend on the abovementioned genetical base of the material. For this reason I doubt that varieties with different genetical backgrounds — lines, families, synthetics e.g. were comparable according to "individual amplitude". For this reason I doubt the validity of this criterion, when an optimum sowing period is to be determined.

Further, I don't recommend the use of the term "early" or "late" in the given meaning, as they usually denote varieties with a short or long period from germination to ripening.

As to the "poppy experiment", it is clear that the shortest individual amplitude corresponds with the optimal technological quality, but I doubt that such a statement can be generalized.

In agreement with the author I would underline a danger of misleading conclusions drawn from one single experiment. I do not think periodical sowing is the solution, but rather a series of experiments, repeated in several years or places, or best of all in several years and places. The solution of such complex experiments is no longer a problem when modern computer techniques can be used.

Let me summarize what has been said. When omitting some smaller misunderstandings in technical and experimental terminology, I must say that this is an interesting new look into the problems discussed. But it can only be regarded as a supplementary method for producing rough and ready information and not as a substitute for proper experimental series.

J. Rod.

Institute of Agrotechniques,
— Hrusovany by Brno,
Brno, Pellicova 5, ČSSR

WHAT ARE THE PROBLEMS IN THE DEVELOPMENT OF FIELD EXPERIMENTS?

Field experimentation, as one of the ways of carrying on agricultural research, plays an important role in the development of crop production. Its techniques and evaluation methods are well established and have recently been improved mainly in mathematical relations. Nevertheless, in many aspects problems have arisen too. So difficulties are caused by the fact that, due to the rapid rate of development, the extent and ratio of the factors of production change rather frequently. Multifactorial experiments would be suitable for analysing this process, they require, however, too much time compared to the rate of development. Another reason is that the mechanization and chemization possibilities of these experiments are far below the level of field production. Since the experiments even today require human work demanding great attention and very often heavy physical labour performed under exposed conditions, their realization will become more and more difficult. Therefore, any initiation promoting the evaluation of the results of experiments and possibly the development of research too, is welcome.

In his paper "Experiments with the horse-race method or without" György Mándy raises some fundamental problems of field experimentation, first of all, in relation with the variety trials. He arrives at the final conclusion that compared to the experiments of 2—3 years duration fractional sowing enables us to develop a longer ecological series, which, under Hungary's weather conditions highly changeable in time and space, may give a more substantial help in evaluating the treatments and varieties. A new idea of the paper is the utilization in variety trials of the phenomenon the author calls individual amplitude, which — in his opinion — well characterizes how much the individual life processes of the plant have taken place under conditions appropriate to the variety.

I think, an analysis of the question relative to vegetables will underline professor Mándy's opinion. This group of plants has the peculiarity of satisfying a manifold human demand, therefore besides the quantitative indices of the yield, the qualitative indices too play a highly important role in the evaluation of the variety. In addition, the seasonal and continuous fulfilment of demands for certain vegetables has always been especially important; this can be realized partly by growing different varieties, partly with repeated production — often through fractional sowing. With the introduction of once-over mechanical harvesting fractional sowing has recently become even more important. Namely, a better utilization of the expensive harvesting and processing machine systems may be an important component of economical production.

Last but not least, I should like to emphasize that the rapid change of varieties does not make it possible to study the value of a variety for a longer time. A considerable part of the vegetable varieties is only kept in production for 4—5 years. It may happen, therefore, that after 2 years of experimentation we already have to decide whether or not a variety should be introduced in commercial production. The situation is made even more difficult by the fact that the agrotechnical and site requirements of the variety also have to be characterized for the purposes of production without sufficient time available for studying such aspects of the plant requirements.

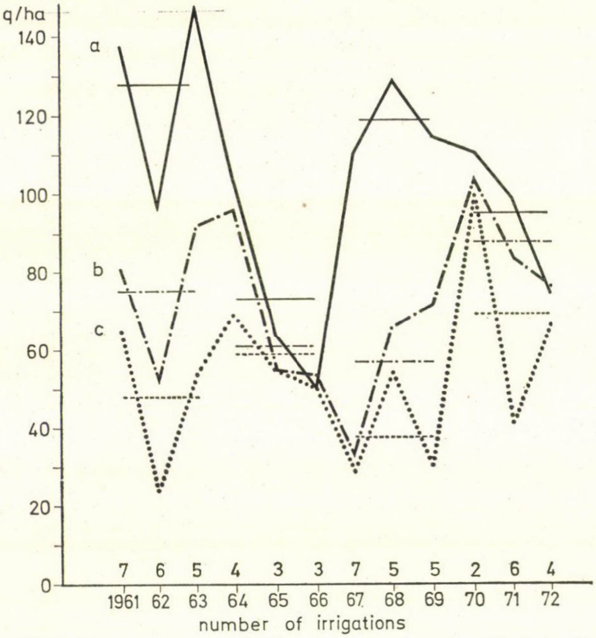

Fig. 1. First-class yield of cucumbers of different water supply. Variety: Delicatess. Gödöllő, 1961—1972. a) regularly irrigated (water capacity 70%); b) irrigated once; c) not irrigated

Professor Mándy is right in stating that in 3 successive years of the variety trials different varieties may be considered the best. This often occurs even in the case of varieties properly included in groups and experiments of similar demands. The situation is no better even if in 3 successive years of similar meteorological type the varieties show the same order of value, because in a subsequent group of years of different meteorological type other varieties may occupy the first place. Examples could be given for this from the field of the variety trials too, still I should like to prove this statement with our own irrigation experiments. I refer here to the results of our irrigation experiment carried on since 1961 with the same cucumber variety under identical conditions (identical sowing time, soil, nutriment supply, spacing, etc.). In this experiment only the water supply of the plants changed, partly according to the weather conditions, partly as a function of the treatments. This paper presents the amounts of first- class crop harvested in the control, from the treatment irrigated once with 40 mm water, and from the plot regularly irrigated at 70 per cent water capacity of the field. In the latter case the number of irrigations indicates how many times the moisture content of the soil decreased to the 70 per cent level, i.e., how many times irrigation was needed (Fig. 1).

The figure shows a high fluctuation of the yield over the years in the above treatments of the experiment. On the basis of the amount of crop per treatment the years can be arranged in characteristic groups. So the cool, rainy years of 1965 and 1966 can be included in the same group, and the warmer and drier years of 1961 and 1968 in another. But even if we mechanically average the results every 3 years, we obtain for this 12- year- period characteristic year groups in the given case. On this basis we may arrive at a different conclusion as regards the necessity of irrigation too. Judging from the crop result of 1961, 1962 and 1963, we may consider irrigation within the framework of the given technology unanimously necessary. If, on the other hand, we take the next three years (1964, 1965 and 1966) into account, irrigation seems uneconomical and consequently omissible. In the case of variety trials this means that a variety studied in a rainy year group will be excluded from the assortment, although it might be very successful in the subsequent years.

Unfortunately, in the field of variety trials I do not know any method that would at least partially solve this problem. Therefore, the author's statement that the lowest value of the so-called individual amplitude of the different development phases of a variety well characterizes the optimum fulfilment of demands in the given development phase of the plant, deserves in any case attention, and a thorough study with a view to its application. A detailed analysis is required in the first place to clarify what it is we mean by the optimum demand of a plant. Namely, this may considerably change in relation to the different production purposes. Let me refer again to another experiment of our own. (Table 1). This work has been carried on

Table 1

Average characteristics of tomato yield
(Mean values of 1964—1971)

	Mean values of treatments showing each year the highest		
	Refraction percentage	Average fruit weight	Dry- matter yield
Sound yield q/ha	425	555	589
1000 fruit/ha	765	728	857
refraction %	6.4	5.0	5.4
g/fruit	56	77	69
dry- matter yield q/ha	27	28	32
Treatments unirrigated	7	—	1
irrigated with 40 mm water in the first critical period	1	1	4
irrigated with 40 mm water in the second critical period	—	—	1
regularly irrigated at 60 per cent water capacity of the soil	—	6	2

— like the above-mentioned cucumber experiment — from the beginning of the sixties with the semi-determinate tomato variety Kecskeméti 42. When considering the 8- year- period between 1964 and 1971, the irrigation requirements with the given technology seem to be different from the aspects of refraction percentage, average fruit weight and dry- matter yield. In the case of tomato grown for fresh consumption its dry- matter content seems to be of lower importance than the size of the fruit. In such a case the application of large quantities of irrigation water is justified. When producing tomato for processing purposes, the dry- matter content per unit area is the decisive factor, although for the processing industry the refraction percentage of the dry matter is also important. Thus, in this case a lower amount of water should be used. I wish to prove with this that the optimum requirements of plants have to be determined from the viewpoint of production (producer and consumer).

We may raise the question of which line we must follow in making the evaluation of the field experiments more efficient. I think professor Mándy's proposal on the fractional sowing experiments is a reasonable initiation in this aspect. As pointed out by many authors, by fractional sowing we can generally obtain a much wider ecological series within a year — the plants are placed under much more diversified conditions during their development —, than when producing a series of the same length in successive years, by sowing at the same time, on a single occasion each year. I should like to give again some characteristic data of our own experiments.

Since 1964 we have been carrying on a fractional sowing experiment with dwarf bean in culture pots, to find out in the first place the water requirements of plants and the possibility of forecasting the time of harvest. In the experiment the plants are automatically supplied with water, the soil and its nutrient content is practically uniform. The variety — and supposedly the biological value of the seed too are the same each year. With the exception of the role of natural precipitation — since water was given in an artifical way — the differences can thus be caused exclusively by the weather conditions. Under their influence the development rate and productivity of plants, as well as other indices show substantial differences. These differences may be greater within a year with fractional sowing than when results of series sown at the same time in different years are compared (Tables 2, a, b, c, d).

All this shows that fractional sowing carried on over two or three years offers a possibility of drawing much more reliable conclusions — acquiring a better knowledge of the plant — than when sowing on a single occasion each year. Naturally, in the case of plants requiring periodical sowing due to the proper supply of the market or other aspects of production, organi-

Table 2

Characteristic features of fractionally sown bean plants
Variety: Fullcrop
Gödöllő

a) 1965. Series 1—7

Series	Time of sowing	Number of days			Total dry weight of plants g/plant	Transpiration coefficient	Ratio of fruit to stem+leaf in dry weight
		From sowing to emerging	From emerging to flowering	From flowering to harvesting			
1	30 April	16	36	14	16.5	235	10 : 90
2	14 May	9	34	17	19.4	275	14 : 86
3	28 May	10	28	14	17.6	260	10 : 90
4	11 June	9	27	17	19.7	270	12 : 88
5	25 June	7	32	14	19.9	247	11 : 89
6	9 July	8	33	17	21.8	260	13 : 87
7	23 July	8	35	22	18.0	269	11 : 89
Mean		10	32	16	19.0	259	11 : 89

b) 1971. Series 1–7

Series	Time of sowing	Number of days			Total dry weight of plants g/plant	Transpiration coefficient	Ratio of fruit to stem+leaf in dry weight
		From sowing to emerging	From emerging to flowering	From flowering to harvesting			
1	8 May	9	33	17	24.5	240	16 : 84
2	22 May	7	36	10	16.5	300	16 : 84
3	5 June	9	33	16	19.4	310	27 : 73
4	19 June	8	30	13	15.7	387	27 : 73
5	3 July	8	38	15	17.1	410	22 : 78
6	16 July	7	30	19	15.0	389	10 : 90
7	30 July	7	35	26	16.1	230	6 : 94
Mean		8	34	17	17.8	324	18 : 82

c) 1964–1971. Series 1

Year	Time of sowing	Number of days			Total dry weight of plants g/plant	Transpiration coefficient	Ratio of fruit to stem+leaf in dry weight
		From sowing to emerging	From emerging to flowering	From flowering to harvesting			
1964	30 April	13	31	19	14.7	369	37 : 63
1965	30 April	16	36	15	16.5	235	10 : 90
1966	2 May	7	39	17	18.5	285	19 : 81
1967	8 May	7	40	12	17.1	336	21 : 79
1968	3 May	7	36	16	15.2	342	25 : 75
1969	6 May	9	34	16	14.2	290	24 : 76
1970	8 May	10	35	12	12.6	334	22 : 78
1971	8 May	9	33	16	24.5	240	16 : 84
Mean	4 May	10	36	15	16.7	304	22 : 78

zation, machine utilization, etc., the evaluation of the varieties and agrotechnics by this method is indispensable anyway.

To return to another notion in the introduction of my comments, we have to reckon with the continuously increasing cost and more difficult implementation of the small- plot field experiments. Therefore this method of experimentation can only be used in the case of very important problems that cannot be settled otherwise, and other ways must be found to get information about the plants. I think, the so- called individual amplitude suggested by Professor Mándy, as well as other similar indicators, may provide a possibility of receiving an answer to various quantitative, qualitative and harvest- scheduling problems of production by combining the small-plot field experiments with microplot experiments. It is possible that in the microplot experiments only the characteristics of growth and development can be numer-

d) 1964—1971. Series 4

Year	Time of sowing	Number of days			Total dry weight of plants g/plant	Transpiration coefficient	Ratio of fruit to stem+leaf in dry weight
		From sowing to emerging	From emerging to flowering	From flowering to harvesting			
1964	11 June	6	32	17	18.8	413	36 : 64
1965	11 June	9	27	18	19.7	270	13 : 87
1966	15 June	5	32	16	19.2	277	18 : 82
1967	19 June	7	29	12	19.4	393	23 : 77
1968	13 June	8	31	19	19.0	337	23 : 77
1969	13 June	6	34	12	16.7	401	30 : 70
1970	17 June	7	31	16	16.4	357	35 : 65
1971	19 June	8	30	12	15.7	387	27 : 73
Mean	15 June	7	31	15	18.1	354	26 : 74

ically determined, and for the quantity and quality of yield mere tendencies will be given. If, however, a better evaluation of varieties or agrotechnical experiments set up with a definite aim can be attained by this, we have taken an important step forward.

Finally, I believe that in getting closely acquainted with the varieties, substantial help can be offered by the breeders who during the development period of the variety, are able to obtain information on many aspects of its demand. The breeder's information is especially important when placing the varieties in the appropriate agrotechnical and utilization groups. Correct grouping is of common interest, since the capacity of a variety is only able to come into full display among suitable partners.

L, Cselőtei

University of Agricultural Sciences,
Department of Horticulture,
H= 2103 Gödöllő

WHY IS ECOLOGICAL RESEARCH IMPORTANT AND WHAT ARE ITS POSSIBILITIES IN VARIETY TESTING?

I have read Mándy's paper "Experiments with the horse-race method or without"* which contains highly interesting and genuine analyses with keen interest. The many-many years experience and the statistical evaluations made up to the present have convinced us that in experiments carried out at a given site the comparison of various factors cannot give reliable results. One of the many reasons for this is that here we are dealing with living material whose production conditions — ecology — cannot be reproduced and so the results may be different every year. After many years experimentation we may express our opinion with more confidence, however, we can still only speak of tendencies. Owing to the very rapid progress and change of production, experiments of long duration are no longer up-to-date. This applies to the variety tests too, including those studying the effects of variety x agrotechnics, and the multifactorial experiments in general.

György Mándy mentions as an example the experiments performed by Árpád Koltai at a given site of the Agricultural Research Institute of the Hungarian Academy of Sciences, and draws conclusions on the response of varieties from it. In the summary of the paper in question Árpád Koltai himself — who performed the experiment — says: "according to three years- yield averages shown in Table 1 and 2, 7th October proved to be the optimum date of sowing for both varieties."

Even on this basis I think it to be too risky to draw far-reaching conclusions from this experiment. It is obvious that the aim of finding out the most important properties of a variety and satisfying the demands of commercial production as quickly as possible can be best attained by carrying on experiments at more than one site every year, all the more so, as the ecological conditions of different sites are not the same. The author is right in saying that multifactorial experiments in most cases give a more precise answer to a question. These multifactorial experiments are, however, extremely expensive, and in certain cases difficult to apply in variety trials,

In spite of this, when the most important questions are to be cleared up, we aim at setting up experiments with two or three factors (treatments).

As for the wheat varieties, we have been trying for years to give an answer to the most important questions of production (germ number, fertilizer response, winter-hardiness, etc.). We also performed sowing- time experiments with winter wheat in 1966. Besides the varieties Besostaya 1 and Fertődi 293, San Pastore too was included in the experiment. On the average of five experimental sites the first half of October proved to be the optimum time of sowing in Hungary, irrespective of the variety. It was interesting that San Pastore, an early variety of poor winter-hardiness, differing in type from both Besostaya 1 and Fertődi 293, gave lower yields when sown either earlier or later than 10th October.

As regards the time of sowing, the so- called "peasant" experiences of many decades have been proved relevant by the most up-to-date experiments.

It can be no objective of the variety trials to find out how much the yield decreases when sowing is carried out late in November. In this case the yield of wheat in general, rather than of the individual varieties, will be reduced compared to yields attained by early sowing.

These trials cannot satisfactorily decide the relative value of varieties.

I should like to call the author's attention to the fact that his conclusions drawn from the sowing dates of 7 and 14 October 1965 are worth supervising. Namely, in my opinion the yield differences were caused in 1965 by the fact that precipitation between 10 and 20 October was neary four times as much as in the previous period, which had an extremely unfavourable influence on the quality of sowing and density of stand.

The author mentions several examples of different crops showing different order of variety. To support his statement he ought to have published the reliability calculations of the differences as well; in this case he probably would not have seen his statement proved.

It is not surprising that the order of varieties shows — in most cases not significant — changes from year to year, since it is living material we are dealing with here. Any parallel drawn between variety tests and the "horse-race method" is incorrect if only because in the case of a horse only its racing ability and not the horse itself has to be reproduced. At the same time, the seed of a variety is reproduced every year. Since the conditions of reproduction (breeding for variety maintenance, ecological conditions, etc.) also determine the yielding ability of the various varieties, it is obvious that the yield varies from year to year. Naturally, the yearly changes of the ecological conditions also have a part in establishing the varietal order. To avoid possible misunderstandings, there is no question of my rejecting the principle of ecological experiments proposed by the author; we only want to put everything in its right place.

S. Kapás

National Institute for Agricultural
Variety Testing
1024 Budapest
Keleti Károly u. 24.

IN THE EVALUATION OF VARIETIES, CAN THE NUMBER OF EXPERIMENTAL SITES OR EXPERIMENTAL YEARS BE DEMONSTRABLY REDUCED BY PERIODICAL SOWING?

The results of field experiments have two basic requirements: they should be reliable and easy to generalize.

Reliability depends on the conditions of the experiments and on the exactness with which they are carried out. The statistical evaluation reveals whether or not the differences between the treatments are reliable. Owing to the faults of execution and deficiencies of environmental conditions, it frequently occurs that the result is not statistically justified, or is only suitable for pointing out great differences. An increased number of replications helps — to some extent — in eliminating such difficulties.

Since, in the case of field experiments, such difficulties arise even when the first requirement is to be met, it is easy to understand that the fulfilment of the second requirement encounters still more difficulties. It is a general opinion that the results of field experiments are — in most cases — only valid for a given place and year. Conclusions of general validity for larger areas or more than one year can only be drawn from serial experiments carried on for several years at a number of places.

The general character of experimental results can be promoted by other means too. One of them is the method of periodical sowing recommended by György Mándy. In this case the same changes of the ecological factors find the experimental object in different phases of development and growth. It is very nearly a case of meteorological factors of more than one year acting simultaneously, or of climatic conditions of different regions prevailing at the same place. It is rightly supposed that this contributes to the general character of the results, but we do not know to what extent.

The ecological conditions can be modified by other means too. Different quantities of seed sown, different ways of soil preparation, various rates of fertilization may also serve the same purpose. Such interventions have to be carefully considered, as they are expensive, and no reliable data are available whether — and to what extent they can replace experiments carried on at more than one place over several years.

This doubt is not decreased even by the author's example. After a closer examination the data of the experiment reported by Koltay (Table 2) give a very odd picture. With the rather high value of significant difference left out of consideration, according to the data of yields obtained with different sowing times Fertődi 293 was superior in six cases, and inferior in only three cases to Besostaya 1. If only the yields obtained with optimum sowing times (30 September — 21 October) are compared, Fertődi 293 was better in three cases and worse in one case. When we examine the averages of the treatments in the three years of the experiment we find Fertődi 293 to have been superior in two years and inferior in one year to Besostaya 1. Finally, if we compare the averages of all treatments in the three experimental years, we again find Fertődi 293 to be the winner. From the above we ought to draw the conclusion that of the two varieties Fertődi 293 is the higher yielding and more reliable one. However, the data of the national variety trials carried on over many years and at many places have made it obvious that Besostaya 1 is the one of the two varieties that gives higher and more reliable yields, as confirmed by the large-scale practice too. Thus this periodical sowing experiment did not help in generalizing the results.

György Mándy is right in saying that the general character of the results of field experiments ought to be improved. So far, this aim has only been attained in one reliable way: with experiments of the same pattern carried on at more than one place, over a number of years, with several replications. This is not a cheap method either, and is only suitable to point out greater differences. Periodical sowing would increase the expenses, and it is a question whether the result could be generalized in a shorter time.

Carefully planned parallel experiments are required to decide whether the number of experimental sites or experimental years can be reduced either by periodical sowing or by any other method. If it proves true, the proposal is very useful. The author will certainly give experimental evidence of his trials gained in many years' biological studies.

J. Lelley

6721 Szeged,
Vidra u. 1.

DOES THE DUTY TO EXPLORE THE ECOLOGICAL DEMANDS OF VARIETIES APPERTAIN TO THE PLANT BREEDER OR TO THE ORGAN PERFORMING THE QUALIFICATION OF VARIETIES?

In his extremely interesting discussion professor Mándy enumerates further convincing proofs concerning the ecological investigations of varieties initiated by him. This time he relies not only upon his own experimental results, but supports the extraordinary efficiency of forming "ecological series" in variety trials by other authors' numerical data, too. With his method, well- known from the literature, some characteristics of varieties in certain species remaining hidden under normal production conditions can undoubtedly be disclosed, so the effect of the ecological demands of varieties on the manifestation of certain economic properties is obvious.

I think, professor Mándy's views of the reform of variety trials do not in the first place concern the qualification of varieties and the state variety trials. It is the plant breeder, first of all, who has to perform such analysing and exploring work on his own prospective variety, and report its final results to the variety- qualifying organs in the form of well- defined special requirements. Namely, no research activity of this kind can be expected from variety qualification, if only for financial and technical reasons, since both the area and the number of treatments would suddenly increase manifold. At the same time it must be taken into consideration that experimentation would be reduced in time, and thereby also made cheaper, by the ecological method, and in any case the data of traditional experimentation would be usefully completed by it. Therefore, we must think it over seriously whether such ecological series should not be set up for some main crops parallel with the state trials too, possibly with plants suggested and selected by professor Mándy. In theory the breeder has always had the possibility of making reservations concerning the sowing and harvesting time, amount of seed, etc., of his prospective variety, but — owing to the great risk involved — the breeders have but seldom made use of it.

Finally, as for the term "horse-race", we only want to note, that it is in the very case of introducing the method recommended by professor Mándy that we can rightly use the term, since starting different- capacity horses at various distances to compensate for the handicap is analoguous with taking the different ecological demands of varieties into consideration.

I. Bócsa

Research Institute of Plant Growing and
Soil Conservation, Kompolt

CAN THE DIFFERENCES IN ENZYME FUNCTION BETWEEN GENOTYPES BE EXPLAINED BY THE QUALITATIVE AND QUANTITATIVE DIVERSITY OF ISOZYME COMPONENTS?

The most recent research results of molecular biology and ultrastructure render it possible to better understand the most complex science, ecology. According to Prof. Zoltán Szabó's brief definition: "The genotype predestines, the peristasis realizes". Modern ecology proves the validity of this thesis with the very results of molecular and population genetics.

Mándy's ecological researches on cultivated plants are generally known, and his conclusions serve the practice directly. Hence, the new problem raised by him is welcome, since it concerns a very important question: variety qualification, that is, the "judgement of varieties". The present method of variety qualification really cannot be considered reasonable. The "horse-race"-like starting may give an opportunity for drawing incorrect conclusions, so the method is not only slow (it is not "motor racing"), but involves errors as well. The traditional method is especially unreliable when chemical properties deciding the quality are also analysed. In this way we may arrive at such incorrect chemotaxonomic conclusions as suggesting that a certain compound is, or is not contained in the plant examined. Besides the reactivity of the chemical method, the ecological effects have a decisive part, and the latter can only be correctly judged by a careful ecological surveying. From this the complex ecological survey based on periodical sowing — as recommended by Mándy — is a very good method even from a practical point of view.

It follows from the above that in the work of breeding important results can only be obtained (especially in Hungary, with the particularly extreme climate of the Carpathian basin) if — knowing the possibilities given by its genotype — we study a cultivated plant in a number of "ecological series": in experiments laid out with periodical sowing — as repeatedly recommended by Mándy. Plus variants thus evaluated can be used for further breeding purposes.

From a theoretical aspect the method elaborated and suggested by Mándy may give the possibility of drawing further conclusions. For this the correlations of genom, isoenzyme spectra and ecological reaction present a basis.

It has been proved by human biological researches that a "gene defect" — often only an incorrect base coupling brought about by the tautomeric change of a purine — or pyrimidine base — may induce a permanent change of enzyme structure and a pathological function. These researches also promoted the organic chemical research in this direction. It is due to this that we are getting to know more and more about the quaternary, spatial structure of the enzyme molecules too, whereby it becomes possible to interpret the relation of the changed enzyme structure and function from an isoenzyme aspect as well.

From a phytobiochemical point of view the knowledge of enzyme structures and biosynthetic turnovers gives a new meaning to the role and functions of isoenzymes. Since a given genotype has a definite isoenzyme spectrum, differences in enzyme function between the genotypes can be explained by the qualitative and quantitative differences of isoenzyme components.

Hence, the different individuals of any stand of a cultivated plant variety can be characterized by different isoenzyme quantities. As an increasing number of evidences — including our own germination biological investigations too — confirm that the individual isoenzymes of the spectrum of enzymes performing the same function (e.g. peroxidase, phosphatases, amilase, etc.) can be characterized by different temperature optima, the ecological reaction of the stand of a given variety becomes interpretable, first of all as regards average temperature and heat amount.

It is in this way that the enzyme-biochemical researches make it possible to explain the responses of cultivated plants to the ecological conditions in molecular biological respects too.

L. Gy. Szabó

Research Institute for Forage Crops
Iregszemcse

ARE THE MULTIFACTORIAL LONG-TERM EXPERIMENTS REMUNERATIVE WHEN EVALUATING PLANT VARIETIES?

On the basis of analysing the results of experiments carried on for several years in Hungary, the paper arrives at the conclusion that the production value of varieties or treatments obtained in experiments set up with a single sowing time a year may be quite misleading. To avoid drawing false conclusions, the paper thinks it desirable to carry on so-called ecological experiments, with different times of sowing rather than starting the variety, fertilization or other experiments at a single sowing time (horse-race method).

In field experimentation methodologically well-proved arrangement and evaluation methods are used to perform these so-called bifactorial experiments. And if beside the variety and sowing time the effect of different crop years is also to be taken in consideration — as reflected by the data of Table 2 in the publication —, the number of the examined factors will be three. But in a proper ecological experiment information ought to be obtained — beyond the mentioned factors — about the effect of the habitat too. With this we have arrived at a polyfactorial (a × b × c × d type) experiment. If each factor is only studied in three stages, then the number of treatments will be (3 × 3 × 3 × 3 =) 81. And if we wish to set up all treatments with 3 replications each — as the significance calculations require replications —, then we have to arrange the precise sowing, care, observation, harvesting, data collection and expert evaluation of a total of 3 × 81 = 243 plots. The systematic implementation of such a multifactorial, so-called long-term experiment series requires a thorough organization and considerable funds. It is thus worth considering when and for what purposes to start such experiments.

It is necessary to emphasize this because the publication that recommends the ecological experiments to replace those carried out with the "horse-race method" does not mention the above conditions. Nor is the reliability of the examined actions and interactions clear from the presented data — except for Table 2.

From a single parameter (i.e. from the mean values without knowing the variances) no useful conclusion can be drawn for practical purposes. This is especially impossible when the observed differences are very small as is the case with most of the presented data. In this case our conclusion confirms at the best the well- known results of a many years' plant growing practice. Such a statement is e.g. that the optimum sowing time is the first half of October for winter wheat, and the first week of May for sunflower. As to the latter, we have to note that the sowing time-dependent decrease of the amount of precipitation shown in Table 2 does not by any means indicate the lower water requirement of the plant, since plants sown in the first decade of May and optimally developing in a soil of adequate temperature can utilize for their development not only the amount of precipitation falling after the sowing, but also a part of that having fallen before sowing and stored in the soil.

We can agree with the publication in its statement that the ecological effect can be assessed by the scatter of development phenomena within the stand. This cannot, however, be characterized by the time interval between early and late plants alone. Namely, to express the ecological response of the stand frequencies pertaining to the values between the extreme values must also be taken in consideration besides the individual amplitude. On the other hand, the widening of the individual amplitude may be caused by many factors other than the sowing time. For example, in the case of plants sown into loose, dry, cloddy soils not only the germination amplitude will be wider but also that of the later phenophases. With all this taken in consideration, the individual amplitude, or even more so the individual standard deviation (or its square, the variance) can well characterize the response to a given environment of not only the varieties but also the sowing times and even the stands of certain years, and the reliability of the effect can be statistically controlled with the F-test. It would be interesting and useful to process and evaluate, e.g., the data of Table 3 and Figs 3 and 4 with the above taken in consideration.

The opinion expressed in the last section of the publication that in the variety trials and other experiments the "horse-race method" ought to be given up and the effects studied, instead, in ecological experiments, can generally be accepted, however, with the following points taken in consideration:

Prior to introducing the new Hungarian or foreign varieties or agrotechnical procedures in commercial production, it is advisable to get acquainted with their ecological responses in well- organized and methodologically properly conducted multifactorial experiments. In these experiments information is optimally given not only on the final results of the quantity and quality of yield, but also on its components, reliability, as well as economic efficiency.

It is only on the basis of such reliably demonstrated actions and interactions that the special ecological requirements of new varieties can be taken into account in later experiments, or in the course of introducing them in the practice. The realization of actions — so the action of sowing time too — pointed out without knowing the significance, is uncertain, and often may give unexpected negative results. Besides the already mentioned methodological difficulties experiments started at different times may have other disadvantages too. Parallel with a shift in the sowing time, of the ecological factors the length of the daily illumination changes in the first place (increasing in spring and decreasing in autumn) regularly every year. The other factors — such as light intensity, soil and air temperature, and particularly the amount of moisture in the different years follow the effect caused by and expected in advance from the different times of sowing less regularly. All these, mostly unforeseen changes considerably influence the soil structure, and thereby the germination and the subsequent initial development, furthermore, they have an effect on the tending, protecting and harvesting operations to be carried out during the vegetation period. The optimum time of sowing can thus be determined with an accuracy of ± 1 week at the best. If we add to all this that beyond the optimum biological requirements the operational and work- organization aspects are also important in choosing the time of sowing, it will be clear that the individual development control by changing the time of sowing is only possible within certain limits.

The publication has the undeniable positive feature of calling the attention of the reseachers to studying the ecological requirements of the varieties in this way. However, in the course of further researches the above- mentioned methodological principles ought by all means to be asserted.

J. Füredi

University of Agricultural Sciences
Department of Plant Breeding
2103 Gödöllő

IN PLANT PRODUCTION IS IT NOT POSSIBLE TO GO BEYOND THE GENETIC POSSIBILITIES OF A VARIETY, EVEN WHEN THE MOST OPTIMUM CONDITIONS HAVE BEEN PROVIDED?

I often meditated on the question why the statement that the genetic possibilities of a variety cannot be overstepped in plant production, even by ensuring the most optimum conditions sounded so well in lectures. What are these optimum conditions and what is their extent? It sounded doubtful to me (and still does) that the productivity and ecological optimum of a plant can be expressed in the antropocentrically simple way it usually is: in tons and centimeters, with only the yield kept in view. The yield and the comfort condition of a plant are in correlation, but this does not necessarily mean that the terminology of a farm economist and that of an ecologist are identical. The questions raised in the paper can perhaps be reduced to these two aspects, and subsequently I should like to have a closer look at them. Let us begin with the possibly simpler view of the farm economist.

In the variety trials we start the varieties studied at the same time, and take the one giving the highest yield for winner. The yields of stands sown simultaneously (replication in space), however, indicate in the first place the occasional soil deficiencies. If the "horse-race" takes place on a larger area, or possibly on a national scale, besides the soil factor even the simultaneous start may be doubtful, e.g., due to local rainfalls. If the horse-race is carried on for at least three years (replication in time) we may obtain a practically, or even theoretically acceptable index for the productivity of the variety. Besides the problems and faults mentioned by Mándy the main deficiency of this trial method lies in the antropocentric view: the start is determined by the breeder, or in an even more extreme case the producer (coincidence of expertness, labour force mechanization and weather).

Periodical sowing substituted for the annual periodicity gives a much more rapid and exact result, when the plant is studied as a system interrelated with its surroundings. A five-phase ecological series — if well chosen — may be equal to a five-year annual horse-race. The method whose importance has been emphasized by Mándy for many years will only be fruitful if we try to decode the behaviour of the variety. And here we have arrived at the second, ecological view mentioned in the introduction.

The breeding practice — quite naturally — has been selecting for characteristics useful from the point of view of man (yield, height, compounds, more rapid growth, etc.) and stabilizing these characteristics for thousands of years. Natural selection, on the other hand — in the original evolutionary sense — favours the selection of adaptive individuals. The variety maintenance and propagation, the monoculture, the closed system of farming, requiring an increasing acreage, acts as a reproductive isolation and involuntarily inhibits the gene infiltration. The natural population (and the gene pool belonging to it) is a dynamic unit which in the modern agricultural practice becomes more and more conserved. The consequences are well-known: sensitivity to sowing time, decreased resistance to pathogens, poor gene content, etc. The damage arising in this way is greater and more unnatural than that involved by the application of pesticides which can be withdrawn overnight from use, while the changes caused in the genes might be irreparable. The individual amplitude introduced by Mándy successfully characterizes the ecological flexibility and comfort state of a population: it is not an antropocentric unit of measurement but the measure of homoeostasis with the environment.

In a given place (uniform soil) and not too long ecological series (to exclude the problem of photoperiodicity) sowing time is the only variable depending on us. Thus, from an information- theory point of view, weather is the source, or transmitter and the plant is the receiver. For the transmitter the receiver is perfectly indifferent, this is how it differs from human or animal communications. For the plant weather — being a concept —

does not exist, but there is a mass of more or less regular signs ultimately generated by the sun and the rotation-revolution of the Earth. It is to these series of signgroups that the plant has adapted itself during thousands of years, that is why through its special enzyme system it is able to select and decode as an appropriate "message" the series suitable for the species or variety (genetic nature). The realized message is the phenotype. The transmission of the information may be disturbed by some noise, e.g. extreme cooling; by the transmitter and receiver getting out of tune, e.g. extreme sowing time, sensitivity to sowing time. As a consequence of the noise the original message will be modified, in some cases to such an extent that it cannot be coded (becomes meaningless), and the plant dies. Under natural conditions the noise is most easily realized by periodical sowing, the extent of noise can be measured by the variability appearing in the receiver (variety). The broad-band, more balanced individual amplitude of a variety (population) suggests a higher adaptability, ecological flexibility. (The question of reliable yields, and more risky high yields, respectively.)

For us, on the other hand, the phenotype, behaviour of varieties set in an ecological series is a genetically generated mass of signs (message) which has been modified by the ecological factors (like noise). For this reason in the receiver (at us) variability is likewise expected (naturally on a conceptual level), until the common language of the plant and the special branch of science develops according to the rules of coding – decoding. I consider the term "individual amplitude" to be an appropriate special term, as it concentrates the relation of a population to the environment (the comfort state) in an easily measurable concept.

Finally, I should like to add; it is a good thing that the Acta Agronomica has published Professor Mándy's paper. I think the Forum will promote the objectives set by the Hungarian Academy of Sciences (General assembly: Szentágothay) in the field of researches on the higher levels of organization hierarchy.

G. J. Maácz
Agricultural College,
4401 Nyíregyháza

WHAT IS THE SHARE OF CROP PRODUCTION ON THE ONE HAND, AND OF BREEDING, ON THE OTHER, IN THE YIELD INCREASE?

It is hardly questionable that our small- plot and field- experiment techniques are obsolete and need modifications in many respects. Mándy recommends, in essentials, the well- known method of periodical sowing completed by his own ideas and technology as a better way of acquiring a thorough knowledge of the varieties and solving the examined questions.

There is no doubt that by sowing repeatedly, at different times, we are given a higher possibility of getting acquainted with the properties of plants. At the same time, the most important questions cannot be answered as yet even by this method.

Namely, apart from its yield, adaptability is perhaps the most important character of the variety. However, a variety has to adapt itself not only to the time of sowing, but to a range of other factors too, from the personal habit of the researcher to the different chemicals.

Let us enumerate some environmental factors influencing — at least indirectly — the yield of the plant material included in the experiment. They are plot size, arrangement, number of replications, sowing time, sowing machine, soil conditions, nutrient supply, climatic factors, epidemic diseases, chemicals used in the treatments, other crop- production factors, day length, amount and composition of light, height above sea level, degree of latitude, etc.

To obtain a comprehensive picture of these factors, Borlaug e.g. sows his experimental plot twice a year, at sea level and several thousand meters above sea level, respectively. The more or less developed breeding material is then tested in at least two dozens of experimental sites, in various countries. In our own way we do the same when testing our material at least at five experimental stations in Hungary, where a special opportunity is given to expose the plants to infection, irrigation, winter frost, etc. The prospective varieties are systematically tested in 25—30 farms simultaneously in the case of wheat, and about 100 farms in the case of maize. These methods are undoubtedly more suitable for assessing the potential productivity than the traditional "horse-race method".

I should like to mention a single example to show that we cannot consider a variety's genotype apart from its environment. The basic factors of increasing yield averages have been studied by a great many authors in different ways. One of the most frequent classification methods compares the influence on the yield obtained as a result of breeding with the improvement of agriculture attributable to the crop production factors. For example, when taking the yield increase for 100, the result of the breeding work is 30 per cent, and that of crop production 70 per cent. It often occurs that the agrotechnician examines the new variety under the earlier and recent conditions of cultural practice and regards the yield difference as an effect of crop production.

Only if we were to study the effect of crop production and the effect of the genetic change in a new variety in a, so to say, "diallel" way, could we determine how much yield variation to attribute to either the genetic changes, the improved crop- production methods or the interaction of these two factors. All possible variations are;

1. Old variety, old crop production methods
2. Old variety, new crop production methods
3. New variety, old crop production methods
4. New variety, new crop production methods

We have not seen such an experiment, but in India under non-irrigated conditions the Mexican wheat varieties gave lower yields than the old Indian varieties. It was only under irrigated conditions and with a good nutrient supply that they gave twice to three times higher yields than the old varieties. In this case what is the share of crop production on the one hand, and of breeding, on the other, in the yield increase? Is it possible to tell?

The breeder's dilemma

The breeder wants to know many genotypes in a short time, since his work consists, in essentials, of separating the good from the bad. This work can hardly be completed with a single experiment. It is probable that by the method of periodical sowing we would get closer to the nature of adaptability, but in this case each variety ought to be sown in 20—30 replications instead of the usual 4—6 every year. It is questionable whether this is possible.

The demonstration of ecological elasticity needs exposure to extreme conditions. This might mean investigations at an optimum and a far below optimum level. These optimum and minimum conditions can be created by periodical sowing, denutrition, chemical treatment, or possibly by changing the day length or light conditions, and naturally in many places by simultaneous examinations and in other ways.

In our experiments we have begun testing the strains, their hybrids as well as the varieties used for producing wheat varieties by the so-called "stress incorporation" method, in which the behaviour of plants is studied with the minimization and optimization of certain factors. These miniature plot experiments are aimed at selecting the plants for adaptability. By crossing the populations thus selected we should like to get some more information about the genetic nature of adaptability. The experiments have been carried on for two years only, so it would be premature to draw conclusions from them. Anyway, we think that they represent a way by which some of the deficiencies of field experiments can be eliminated.

In our opinion the importance of Mándy's paper lies mainly in its calling attention to some deficiencies of our obsolete experimentation techniques, encouraging us to seek new ways better than those used so far.

Z. Barabás
Cereal Research Institute
6726 Szeged

IS A HEAT- INDUCED SPECIAL PLANT- HORMONE INTERRELATION THE BIOLOGICAL BASIS OF THE OPTIMUM SOWING TIME?

According to the results of many years experiments carried on by Mándy with a number of cultivated plant varieties, the minimum individual amplitude determined in fractional sowing is in close correlation with the optimum sowing time of the variety concerned. The minimum individual amplitude of any variety can even be detected in a single year fractional sowing experiment and is in closer relation with the temperature than are the optimum development and maximum value of the components with the precipitation. On this basis we seem to be justified in pointing out that the optimum sowing time and the related influence of temperature play a very important role even in the early phases of vegetative development, and through them in the favourable development of the generative organs which can be regarded as fundamental biological determinants of the varieties' productivity. The close correlation between the favourable influence of temperature and the minimum individual amplitude can supposedly be brought into connection with a morphogenetic induction of the optimum isozyme spectrum and the generative organs. In the phenomenon of thermoperiodicity, beyond the effects of the temperature degrees, the heat amounts acting in the individual phases of development — and the maximum temperature fluctuations, respectively, too — have a highly important part in the morphogenetic induction of the vegetative and generative organs. On the other hand, the optimum productivity of a variety is supposedly in accordance with a definite isozymes proportion which, while genetically determined, is realized through heat induction. The ratio of protein macro- and microfractions (different electrophoretically as well as in molecular weight and intensity of enzyme activity) is determined by the free deoxyribonucleic acid template, which is manifested at the level of the messenger ribonucleic acid and through its biological life time, but, according to the most recent experimental results, plant hormones (hormone-like compounds) are involved in their induction. The intensity and levels of the synthesis of plant hormones (endogenous regulators) are primarily controlled by the changes of temperature and heat amounts, and it is through them that the hormone interrelation in the plant tissues is realized. Besides the temperature fluctuations the varying turgescence of the tissues too exercises a decisive influence on the interrelation of the plant hormones (auxins, gibberellins, citokinins, growth retardants, endogenous inhibitors and activators, etc.), which, in all probability, directly acts on the activization of the deoxyribonucleic acid and through this on the synthesis of the appropriate messenger ribonucleic acid, and thus on the isozyme spectrum. Supposedly, it is through such a mechanism that at the stages of germination and early development the optimum productivity of a variety is realized through the generative induction, in the first place in temperature and temperature fluctuation effects. It is highly probable that the generative induction already takes place during the early vegetative development, or at least the first determinative steps of the multigrade effectivity are fixed, but without an initial optimum impact maximum productivity cannot be realized. The results of multilateral and multifactorial ecological comparisons of varieties naturally agree with the views of modern molecular biology, due to the general conclusions that can be drawn from the large number of data.

B. I. Pozsár
Agrobotanical Institute
Tápiószele, Hungary

CAN THE LENGTH OF THE VEGETATION PERIOD BE INFLUENCED BY A PRELIMINARY TREATMENT?

In a fractional sowing series the yield values often change. The fact that of the varieties sown at the same time, in a given year one, while in the next year another variety reaches better results, suggests that in the same periods of the successive years the meteorological conditions shift now to warmer, now to colder values, and these conditions are sometimes favourable for the one, sometimes for the other variety, and at the time of germination exercise either a stimulatory or an inhibitory effect on the different enzymes in the enzyme system. The changeability of the weather has possibly been increased for the last twenty years by the artificial satellites. Thus, to select in advance e.g., the winter- wheat variety suitable for the coming autumn weather, has become even more difficult. If we foresaw the climatic conditions from the middle of September to the beginning of November we could sow the proper variety at the given date, or the planned variety under the proper ecological and meteorological conditions at the optimum time. Thus, in many cases, we ought to take the weather into account when choosing the varieties to be sown.

Germination experiments could be carried out under artificial conditions, at different temperatures, or by changing the temperature conditions in the given period of germination, in order to establish the initial ecological requirements of the varieties. It is probable that, in accordance with the results of such experiments, e.g. under climatic conditions unfavourable for a given variety, the activity of enzymes producing higher crop results could be promoted by a few-days- pre-germination (under warmer conditions for varieties with higher heat requirements). This would eliminate the negative effect of unfavourable meteorological conditions and thereby determine the better crop result.

A better understanding of the first increasing then decreasing yields obtained by fractional sowing, and of the whole complex mechanism, calls for human intervention: in the case of early sowing the seed should be exposed to lower, or decreasing temperatures, and in the same way, when sowing after the optimum sowing time we should expose the seed to higher, then increasing temperatures, thus carrying out a preliminary germination. By this, we could attain that man would not be so much dependent on the weather conditions and could direct his farming activity more reasonably.

Under large-scale production conditions where the period of sowing is protracted, besides the early and late varieties, such varieties ought to be employed which, being less sensitive to the ecological conditions, give optimum yields when sown both earlier and later. This could possibly be extended under experimental conditions as mentioned before, that is, the ecological properties of the varieties could at a certain level be widened by an artificial influence.

Under experimental conditions, naturally, the parallel and fractional sowing of varieties makes the comparison more exact and clear, not decreasing the actuality and positive role of fractional sowing in determining the ecological requirements of the varieties.

To return to the late varieties which give lower yields: is the fact that certain life processes begin in spring not involved with their developing more leaves and showing a lower rate of assimilation due to the late sowing, which would have a positive effect on fruit formation if the same variety were sown earlier. Research work ought to be concentrated on the enzymes which control the beginning of the individual development phases.

P. Gracza

University of Horticulture,
Department of Botany
Budapest

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CHRONICA

PAVEL PANTELEĬMONOVICH LUKYANENKO
1901—1973

On the 13th of June, 1973, like on any other day, early in the morning, academician Lukyanenko left his Krasnodar home to go to his place of work at the research institute four kilometres away; an hour later came the sad news that he had died among the wheats he loved so much. This is what Olga Lukyanenko told us, friends and colleagues, at the end of July at Martonvásár about her father's last hour.

Pavel Panteleĭmonovich Lukyanenko, beyond doubt the greatest and most successful winter wheat breeder of our time, was born on the 27th of May, 1901, at Ivanovskaya Stanitsa in the neighbourhood of Krasnodar, as the eighth child of a Kuban peasant family. We visited his birthplace in the summer of 1969, in his company; the house was like that of any other village peasant. In the first years of the Soviet regime Lukyanenko fought in the Red Army. Having completed his studies at the Kuban Agricultural College in 1926, he spent all his life in developing the science of agriculture and producing new winter wheat varieties. The exceptional talent of the great scientist blossomed out at the State Breeding Station of Krasnodar, later Agricultural Research Institute of Krasnodar, where he worked from 1930 till the end of his life.

During the 43 years of his creative period he developed 17 state registered and commercially produced winter—wheat varieties, of which Bezostaya 1 well deserves its fame all over the world. In the last few years this variety was produced over an area of more than ten million hectares in the socialist countries alone, of which 7 million fell within the Soviet Union and nearly 4 million mostly in Hungary, Bulgaria, Yugoslavia and Roumania. But it has been produced on large areas in non-socialist countries as well. In 1972, when visiting Ankara in the company of academician Lukyanenko, we were informed that in Turkey the sowing area of Bezostaya 1 reached 2 million hectares. In the International Winter Wheat Performance Nursery, where the 30 best winter wheat varieties in the world are tested at 38 experimental sites in 23 countries spread over four continents, Africa, Asia, Europe, as well as North and South America. Bezostaya 1 was for years absolute first exceeding by some 10 per cent the grain yield of the next best variety. There is certainly no winter wheat breeder in the world who would not use Bezostaya 1 as a crossing partner in his breeding programme.

In the spring of 1958, before the state registration of Bezostaya 1 in the Soviet Union, academician Lukyanenko sent a few kg seed sample of the variety Bezostaya 1 to Martonvásár; it was with this sample that the first experiment with this variety was started in Hungary. From the beginning of the 1960's Bezostaya 1, introduced at our instigation formed the basis of the recent upswing in Hungarian wheat production, simply redoubling Hungarian wheat yield averages.

The new varieties of academician Lukyanenko, Avrora, Kavkaz and Bezostaya 2, appeared on the wheat fields of the socialist countries at the beginning of the 1970's.

Winter wheat breeding at Krasnodar is based on intraspecific hybridization and controlled individual selection. For the basis of his breeding work academician Lukyanenko applied — as he wrote in 1966 — the Michurinian crossing principle of geographically and ecologically remote forms, and used the method of repeated crossing of young hybrids with other true-bred varieties. By crossing geographically remote forms more vital and plastic hybrids with broader genetical background can be produced. Academician Lukyanenko carried out controlled individual selection and individual testing of progenies as a means of producing new forms with valuable properties. The real value of Lukyanenko's contribution to the methodology and theoretical bases of the breeding of wheat, our most important bread-grain crop, cannot possibly be assessed yet. In the true sense of the word he opened up new paths in breeding cereals for productivity, quality and resistance to lodging and diseases.

Academician Lukyanenko's activity was fully acknowledged in his country, and beyond its boundaries too. He was Lenin and State Prize winner, he was twice awarded the title of the Hero of Socialist Labour, three times he won the Lenin Order and twice the Order of the Red Banner. He was full member of the Academy of Sciences of the USSR and the Lenin Agricultural Academy. Since 1967 he was honorary member of the Hungarian Academy of Sciences; in June, 1969, during his visit to Hungary, he was awarded the Order of the Red Banner. He was highly appreciated in other socialist countries, too. At the Fourth International Wheat Genetics Symposium recently concluded at the Missouri State University in Columbia, USA, before an audience of nearly four hundred scientists gathered from over the five continents the deceased scientist was commemorated with the greatest recognition by the Australian-born Professor Keith Finlay, Deputy Director of the International Maize and Wheat Improvement Centre (CIMMYT), President of the symposium.

I became personally acquainted with Pavel Panteleĭmonovich in 1950. From then on we met at least once every year. We maintained a regular correspondence and exchange of information, and divided between us the most valuable seed samples obtained through our wide-ranging international professional relations. These connections were further developed by László Szunics, C. Sc. (agriculture), who came to Martonvásár in 1970 and brought along his experiences acquired at Krasnodar where he had been a practising university student over several summers, then for three years as a post-graduate student he carried out experiments in wheat breeding under the guidance of the great scientist.

Pavel Panteleĭmonovich Lukyanenko — through his life-work an immortal scholar for wheat breeding — was an excellent colleague, a true man and a good friend.

S. Rajk

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RECENSIONES

TÓTH BÉLA ÉS MUNKATÁRSAI

SZIKESEK FÁSÍTÁSA

SZIKES FÁSÍTÁSI KUTATÁS ÉS GYAKORLAT MAGYARORSZÁGON

AKADÉMIAI KIADÓ, BUDAPEST

B. Tóth *et al: Szikesek fásítása* (Tree planting on salt-affected areas). Akadémiai Kiadó, Budapest. 1972

Tree planting on salt- affected areas is important for many reasons in countries where alkalization occurs, first, because those settled on salt- affected areas also require a place where they find shade and protection against dry winds and intensive sunshine. Secondly, this protection is necessary not only to the people but also to animals and cultivated plants, in the form of trees planted on pastures, or windbreaks bordering arables and roads, of shelter belts, etc. Thirdly, the value of trees grown on salt- affected areas is not negligible either, especially when they compete with the field crops as regards economicalness.

For the above reasons the book written by B. Tóth and his collaborators is especially useful as it contains the results of experiments carried on for nearly half a century at the Experiment Station for Alkali-Land Forests at Püspökladány.

The work published by the Publishing House of the Academy of Sciences runs to some 25 printed sheets and has a very nice get-up. It consists of ten chapters, three of which have been written by soil scientists: I. Szabolcs, F. Jassó and Mrs. J. Leszták. The material processed by them originates from soil analyses performed on the area of the experiment station, and is an integrate part of the chapters written by B. Tóth on forestry proper. This connection may be exemplary in other respects too, having resulted in a high-level work.

The general part dealing with tree planting on salt-affected areas and alkalization, as well as the chapter written on the history of alkali soils planted with trees give a true picture of the historical background and general implications of the question. The presentation of the details is introduced by a description of the natural conditions prevailing at the Experiment Station. Data on soil mapping as well as those characterizing the physical properties — first of all- the water regime — of alkali soils are discussed in full detail, and on a very high level. These are followed by observations made at the meteorological station and by an evaluation of the water conditions. All these together make a perfect characterization of the site possible.

After these chapters running to about half of the book there are four chapters in which the author gives an account of the results of tree-planting experiments, then on this basis of the possibilities of extension.

The author presents in detail the small-plot experiments aimed at studying the effect of soil technology, physical and chemical amelioration, as well as biological soil preparation on the cultivation of various tree species. This part has the great advantage of giving only outlines and evaluation, thus avoiding a too detailed presentation of the data already known from earlier publications.

Information is given in the same systematic way on the semi-scale and large-scale experiments from which conclusions are drawn concerning afforestation, mixed forests, pastures planted with trees, the cultivation of growing stocks, in relation — of course — with alkali soils.

Beyond describing the experiments, the author evaluates the salt-affected growing sites from the point of view of tree planting, starting from the classical systems and arriving at recent evaluation methods rendering an up-to-date distinction possible.

A generalization of the results attained is served by the chapters and parts which discuss the tree-planting conditions of the region including the Püspökladány Station, and by descriptions of major afforestation processes on salt- affected areas. Finally, the last chapter of the book deals with the planning and implementation of tree planting on alkali soils as a practical application of the previously presented scientific results.

All that have been said so far show that the structure of the book is exemplary, as it starts from the details and advances towards generalization, and the latter is built on a firm basis and can be applied in practice.

The text is excellently completed by 55 figures, the majority of which are genuine photos illustrating the conditions and results of the experiments. Among the literary sources 177 works are listed, mostly citing reports on experiments carried on at the Püspökladány Station, but beside them references are made to Hungarian and foreign publications on the subject.

To sum up, it can be established that the book compiled by B. Tóth and his collaborators is a useful guide for all those dealing with the problem of planting trees on salt-affected areas. It covers a successful period of almost fifty years, thus representing a valuable work of science history as well.

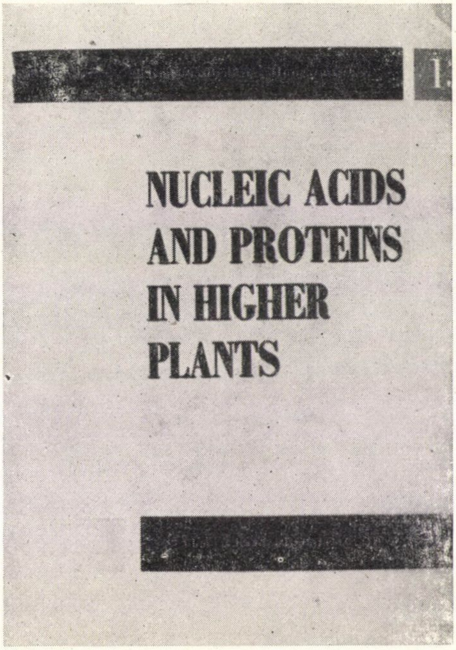
Finally, by summarizing the Hungarian results, the authors have gained scientific and practical distinction not only at home but also abroad.

P. STEFANOVITS

Nucleic Acids and Proteins in Higher Plants (Ed. G. L. FARKAS), Akadémiai Kiadó, Budapest, 1973.

This volume, the 13th in the series Symposia Biologica Hungarica, contains the contributions (all delivered and printed in English) by 37 authors from 16 countries, at a very successful Symposium held at the Biological Research Institute in Tihany, Hungary, from September 2 to 4, 1971.

The reason for organizing this Symposium was the recognition of the need to sum up our present knowledge on plant nucleic acids and proteins from as many aspects as possible. The rapid progress in the study of nucleic acid and protein metabolism in prokaryotes during the past decade has lately had a considerable impact on the experimental approach of the same problem with eukaryotes as experimental objects. It was felt that the time had come to survey the results obtained so far and to pinpoint those aspects of the "nucleic acid and protein problem" which are common in both prokaryotes, and eukaryotes as well as those which are specific for plants. As seen from the list of participants (with full addresses, 9—11), the attendants represented most of the intriguing and up-to-date fields of plant nucleic acid and protein research.



During the Symposium the following topics were dealt with; Section I, Plant nucleic acids (15—111); Section II, Protein synthesis in plants (115—189); Section III, Nucleic acid and protein synthesis in cell particles (193—282); Section IV, Hormonal control of nucleic acid and protein synthesis (285—334); Section V, Nucleic acids and proteins in plant development (337—372).

In Section I part of the lectures covered different aspects of transcription and part of them the structural and functional characterization of DNA and RNA, including plant viral RNA.

R. Julien and Y. Guitton (Perpignan, France) have shown that in the rapidly labelled DNA-RNA complex of radish seedlings the DNA component exhibits a special structure which differs from that of bulk DNA. This special DNA has dC rich sections and utilizes uridine as a precursor, in contrast to the bulk DNA which incorporates thymidine. J. L. Key (Athens, Georgia, U.S.A.) has presented evidence for the occurrence of at least two distinct types of short-time labelled, AMP-rich RNAs (D-RNA, DNA-like RNA: TB-RNA, tenaceously bound RNA) of soybean. The experimental results are compatible with the hypothesis that D-RNA, maybe mRNA and TB-RNA, may derive from the nuclear fraction of plants. B. B. Biswas (Calcutta, India) and co-workers reported on the isolation of two RNA polymerases (I and II) and several protein fractions (A, B and C) from the chromosomal acidic (non-histone proteins of coconut endosperm nuclei. RNA polymerase I has been characterized in detail. Factor B has been implicated as the initiation factor and factor C seemed to facilitate the release of synthesized RNA from the DNA template.

J. M. Bové (Bordeaux, France) and co-workers, in following up their studies on turnip yellow, mosaic virus-RNA synthesis in the plastids, reported the partial purification of a virus-specific DNA-independent enzyme-template complex. A. Kovoor and D. Melet (Paris, France) have shown that growth induction by auxin in explants of Jerusalem artichoke tissue is accompanied by a modification in the reassociation kinetics of the DNA. The first steps in tackling a certainly difficult but hopefully rewarding problem, the methylation of nucleic acids by higher plants, have been taken by M. Abeels (Leuven, Belgium) and co-workers. This is a par excellence attempt to apply to eukaryotes the results of studies on prokaryotes and bacteriophages (role of modification, restriction phenomena). The authors demonstrated the presence of tRNA and DNA methylases in plants and hypothesized on the possible implication of the modification of tRNA and DNA in cell differentiation and development.

Two papers on the methodological aspects of plant nucleic acid research concluded this section. One was by D. B. Dunn and I. H. Flack Norwich, England) on the use of bentonite in the isolation of tRNA from plant leaves, and the other one by F. Solymosy (Szeged, Hungary) and co-workers on the application of diethyl pyrocarbonate in studying the secondary structure of DNA.

In Section II a number of various approaches to study the control and regulation of protein biosynthesis in plants were presented. P. Schopfer (Freiburg, GFR) was concerned with the molecular mechanism of P_{fr}-mediated enzyme regulation. P_{fr}, the active species of the phytochrome system, is an effector of apparent induction and repression of enzyme synthesis. As shown in the lecture, the induction of phenylalanine ammonialyase by P_{fr} is the result of de novo enzyme synthesis. Whereas the P_{fr}-mediated induction of amylase and glycolic acid oxidase activity is a reversible process, involving the functioning of short-lived mRNA (photomodulation), that of peroxidase activity (in the cotyledons) is a virtually irreversible process, involving the presence of at least one stable intermediate (transmitter) in the metabolic chain between P_{fr} and peroxidase synthesis (photodetermination). G. R. Stewart (Manchester, England) reported on the end-product repression of nitrate reductase in *Lemna minor* L. Ammonia and asparagine were shown to act at the cellular level rather than by inhibiting nitrate accumulation. The possible role of non-protein amino acids in controlling amino acid and protein synthesis rather than being merely unusual secondary products, was stressed by L. Fowden (London, England) and co-workers. The role of ATP sulphurylase in the biosynthesis of cysteine in higher plants was studied by J. W. Anderson and W. H. Shaw (Victoria, Australia). ATP sulphurylase, the enzyme catalyzing the synthesis of adenosine 5'-sulphatophosphate, an intermediate in the incorporation of sulphate-sulphur into cysteine, was purified 1000fold and shown to be able to use selenate instead of sulphate as an alternative substrate. This makes the synthesis of selenium analogues of cysteine and methionine possible. This finding is of importance with respect to the metabolism of selenium-accumulator species.

The regulation of protein synthesis at the translational level was also dealt with in several lectures. G. Burkard (Strasbourg, France) and co-workers described some specific tRNA species in bean which are found in the chloroplasts but not in the cytoplasm. These tRNAs are aminoacylated only by chloroplast enzyme preparations and are preferentially synthesized upon the exposure of dark-grown plants to light. In connection with polypeptide chain elongation A. B. Legocki (Poznan, Poland) described an improved method for the purification of two elongation factors (T I, binding enzyme and T II, translocase) from wheat germ and presented some properties of the enzymatic binding reaction in a poly-U-directed system. From their studies on the poly-U-directed synthesis of polyphenylalanine in a wheat embryo system G. A. Lanzani (Milano, Italy) and co-workers advanced the hypothesis that in the above system initiation is operated by uncharged $tRNA^{phe}$. Uncharged $tRNA^{met}$ inhibits polyphenylalanine synthesis probably by competing for the same site, and phe-tRNA synthetase inhibits this process by completely charging and continuously recharging $tRNA^{phe}$, thereby eliminating the uncharged $tRNA^{phe}$.

In Section III most reports were on the role of ribosomes and chloroplasts in protein synthesis. Much less was said about nuclei and even less about mitochondria. U. E. Loening (Edinburgh, Scotland) and co-workers compared the pathways of processing rRNA in leaves and roots of pea. They found that there is a true difference in molecular weight between the precursor of rRNA synthesized in the leaf and that in the root. It is suggested that the difference is due entirely to a small amount (m.wt. about 0.2 million) of excess RNA at one end of the molecule in the root. Possible alternative explanations for this phenomenon at the transcriptional level are offered. The same topic, i.e. processing of rRNA was studied by U. Seitz and Ursula Seitz (Tübingen, GFR) but from a different angle. They followed the appearance of rapidly labelled high- molecular weight RNAs in the purified nuclear and ribosomal fractions of parsley cells in culture, and determined their molecular weights. They found, most interestingly, that the 25S RNA appeared more slowly in the cytoplasm than the 18S RNA. O. Ciferri (Pavia, Italy) summarized the present knowledge on ribosome specificity in protein synthesis in vitro. The results presented support the general belief that mitochondria and chloroplasts are of prokaryote origin. There is, however, one interesting point to note, namely, that mitochondrial protein synthesis appears to be controlled by the nucleus at both the transcriptional and translational levels (experiments with yeast).

F. Parenti (Bari, Italy) and co-workers concluded from their studies on the inhibition by actinomycin D of the light-triggered polysome formation involving cytoplasmic monosomes, that this type of polysome formation depends on the presence of a newly formed mRNA with a low turnover rate. Structural and methological aspects of studying plant ribosomes were presented by S. Zalik (Edmonton, Alberta, Canada) and co-workers and by N. A. Gumilevskaya et al. (Moscow, USSR). The former described the use of sucrose- gradient centrifugation in zonal rotors for the separation of cytoplasmic and chloroplast ribosomes as well as their subunits. A successful reassociation of the ribosome subunits was also reported. The Russian group compared some physical chemical properties of ribosomes from dry pea seeds, cotyledons of germinating pea seeds and pea seedlings. Polyacrylamide gel electrophoretic patterns of the 80S ribosomes and their 60S and 40S subunits were also presented.

In the chloroplast field R. Wollgiehn (Halle, GDR) reported on RNA synthesis in isolated chloroplasts and described some experiments on the characterization of RNA polymerase from chloroplasts, on the determination of the size of RNA synthesized in vitro and on the action of inhibitors on RNA synthesis in isolated chloroplasts. In B. Parthier's (Halle, GDR) contribution the use of different inhibitors was described for the determination of the sites of synthesis of chloroplast proteins. In connection with a special problem, namely infection of plants by obligate parasites, H. Oku (Okayama, Japan) and co-workers have shown that in barley plants, upon infection with powdery mildew (*Erysiphe graminis* f. spec. *hordei*), RNA synthesis in chloroplasts is activated within 24 hours after inoculation. Chloroplast and mitochondrial DNA in pea seedlings was studied by M. S. Odintsova and M. S. Turischeva (Moscow, USSR), using electron microscopy and isopycnic density gradient centrifugation. They found that in both these organelles the DNA was linear in contrast to animal mitochondria in which DNA is known to be circular.

The only lecture devoted especially to plant nuclei was that of C. M. Duffus (Edinburgh, Scotland). She described in much detail and in a highly enjoyable manner a very useful method of isolating endosperm nuclei from immature barley.

In Section IV on the hormonal control of nucleic acid and protein synthesis the first paper was by L. S. Dure (Athens, Georgia, USA). On the basis of the action of actinomycin D and abscisic acid on the activities of two germination enzymes, carboxypeptidase and isocitritase, developmental events during embryogenesis and germination at both the transcriptional and translational levels were suggested. G. L. Farkas (Szeged, Hungary) and co-workers have shown that kinetin and abscisic acid both act on only one (a relative purine specific) nuclease among several nucleases present in the Avena leaf. It was suggested that this very specific response could be used as an excellent system for studying the mechanism of enzyme regulation in plants. L. D. Done (Macomb, Illinois, USA) reviewed the literature on the environmental and chemical control of RNA breakdown in leaves. A very original finding was reported by I. Sziráki and Z. Király (Budapest, Hungary). They demonstrated that a pyrimidine derivative, 6-methyl uracil, was active in six different bioassays for cytokinin, including tissue culture and shoot tip culture tests. This compound can thus be regarded as a typical cytokinin. In the next lecture the interrelationship between auxin content, RNase activity and RNA content in lentil roots was analyzed by P. E. Pilet (Lausanne, Switzerland). He found an inverse relationship between RNase activity and RNA content in different parts of the root. Upon the administration of β-naphthyl-acetic acid RNase activity decreased, whereas RNA content was higher than in comparative controls. By adding isopropyl-N-phenyl carbamate, the auxin level decreased, RNase was activated and the amount of endogenous RNA decreased. The stimulatory effect of indoleacetic acid on RNA synthesis in lentil roots has been analyzed in an elegant way by P. Penon (Marseille, France). He has shown that a short treatment of lentil roots with the hormone leads to an increase in the amount of nuclear uridine-rich RNA, whereas a prolonged treatment causes an increase in adenine-rich RNA containing polysomic mRNA. The possible role of the former in the regulation of gene expression was discussed.

The limitations of our present knowledge of the role of nucleic acids and proteins in plant development are well documented by the heterogeneity and lack of central concept in the lectures presented in Section V.

The role of nucleic acids and nucleotides in the control of plant development was reviewed and documented with some experimental data by E. G. Brown (Swansea, Wales). R. F. Lyndon (Edinburgh, Scotland) made the interesting observation that the slowly dividing cells (the central zone) at the summit of the shoot apex of pea incorporate ^{3}H-uridine more rapidly and ^{3}H-thymidine more slowly than the faster dividing cells elsewhere in the apical meristem. In these experiments the incorporation of labelled uridine and labelled thymidine was taken as a measure of RNA and DNA synthesis, respectively. M. A. Stahman and D. M. Demorest (Madison, Wisconsin, USA) have shown that upon inoculation of plants with selected viruses, bacteria and fungi peroxid ase activity increases and often new peroxid ase isozymes appear. It was suggested that the peroxidase-oxidation products of indole-3-acetic acid or phenols might activate genomes to increase the synthesis of proteins and ribosomes. This Section was concluded by the lecture of T. ap Ress (Cambridge, England) and co-workers who reported changes in the enzymes of carbohydrate oxidation during the differentiation of the root of *Pisum sativum*. Their findings are compatible with the hypothesis that the variation found in the amounts of enzymes determines the cells' capacity to provide the $NADPH_2$ required for biosynthesis during the differentiation of the root.

As can be seen from the results presented during the Symposium the experiments reported covered a very considerable part of modern plant biochemistry. The lively discussions, unfortunately missing from the present volume, greatly contributed to the high standard of this very successful Symposium.

F. SOLYMOSY

GY. MÁNDY: *Pflanzenzüchtung kurz und bündig.* VEB Deutscher Landwirtschaftsverlag, Akadémiai Kiadó, Berlin—Budapest, 1970.

The work is a clearly arranged manual-form book of a size of 336 pages. The excellent translation is the work of Sándor Országh.

After a short explanation of genetic concepts the author deals with 66 crop species in tables laid out uniformly. The species are distributed into nine main groups according to the following:

Cereals: wheat, rye, oat, barley, maize.

Legumes: pea, bean, lentil, soybean, broad bean, white lupine, yellow lupine, chickling vetch.

Root crops: potato, topinambur, beet, carrot, chicory.

Oil plants: rape, turnip, poppy, sunflower, white mustard.

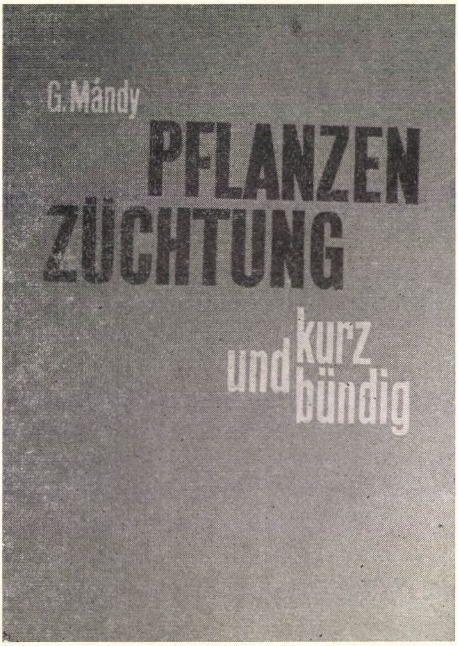
Industrial plants: common tobacco, coarse tobacco, hop, hemp, flax.

Papilionaceous fodder plants: alfalfa, red clover, white clover, crimson clover, bird's foot trefoil, bokhara clover, saintfoin, common vetch, hairy vetch, Hungarian vetch.

Grasses: timothy, perennial ryegrass, Italian ryegrass, meadow foxtail, agrostis, onion couch, cocksfoot grass, meadow fescue, red fescue, common meadow grass, golden oat grass, crested dog's tail, Hungarian brome, wheat-grass, canary-grass.

Other fodder grasses: foxtail millet, Sudan grass.

Vegetables: cabbage, red beet, parsley, celery, radish, onion, tomato, cucumber, lettuce, spinach, asparagus.



The author discusses the most recent knowledge of the various species in the form of tables according to the following aspects: original form; centre of origin; number of chromosomes; chromosome aberrations; cultivation in the world, in Europe and in the German Democratic Republic; related species in Central Europe; interspecific crosses; intergeneric crosses, time of flowering; blooming demand; blooming process; duration of flowering, flowering gradient; method of pollination; fertilization; effect of inbreeding; emasculation; isolation; inheritance of characteristics; mutations; breeding goals; method of selection; breeding method; literature.

The data on flowering biology as well as a compilation of the most important world- and Hungarian literature are especially valuable. The latter includes works on Hungarian cultivated plants.

The excellent summarizing manual published in a language spoken in most parts of the world not only provides long-needed information for breeders, genetists, agrobotanists and practisers of agriculture, but also makes the university education of agriculturists and biologists easier. It is a pity that the work does not discuss the most important fruit-, ornamental- and medicinal plant species. Nevertheless, it provides a thorough and up-to-date information on the most important species of cultivated plants.

L. Gy. Szabó

D. Parkinson, T. R. G. Gray, S. T. Williams: *Methods for studying the ecology of soil micro-organisms.* International Biological Programme, 7 Marylebone Road, London NW1, Blackwell Scientific Publications, Oxford and Edinburgh, 1971.

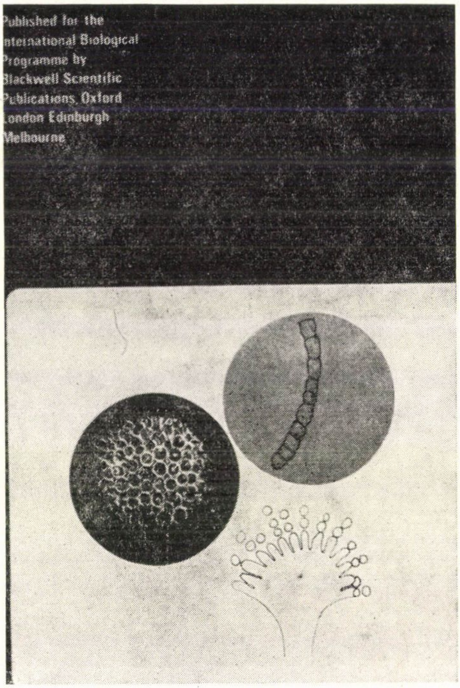
This book is No. 19 in the series of the International Biological Programme (IBP) started in 1964. Two others have already appeared, No. 18 Methods of study in quantitative soil ecology: population, production and energy flow, edited by J. Phillipson sponsored by the Terrestrial Productivity Section (PT), and No. 15 A manual for the practical study of root-nodule bacteria edited by J. M. Vincent, sponsored by the Production Processes Section (PP) and a third one which is under publication: Bacteria of fresh- water ecosystems, all concerning the ecology of microorganisms.

The great and inevitable advance of ecology especially in the last one or two decades and the tendencies of its future development equally reinforce the necessity of having such methodological publications. These help in the investigations of the natural ecosystem — in our case — the microbiological study of soil ecosystems.

The necessity and great importance of these kinds of works have been realized by the IBP by their timely appearance.

The handbook reviewed here is the first of its kind, bringing together the old but well-tried microbiological methods with the latest ones on soil ecosystems, thereby meeting a great demand in this field. It is a comprehensive guide to microbial ecologists who can get valuable information on problems of method at the present stage of knowledge.

According to our opinion, this manual will be useful not only to researchers who are



new to soil microbiology or production ecology, but to experienced workers too, making survey easier. Moreover, it will be most helpful to the specialist working in underdeveloped or less developed countries and places and also it could be a basic handbook for special microbiological courses and teaching.

These are promoted by the fact that it is written in a flowing clear style, is thorough without too much elaboration, with precise well-defined expressions and is easily comprehendable to readers from not- English speaking territories too.

It is also a good basis for enlarging in future editions. Having surveys, comparisons and criticisms, it will stimulate soil microbiologists to modify and improve the methods written down in it and perhaps find new ones. The detailed classification of its topics which enables getting quick information, we think is absolutely indispensable in a work of this kind.

The handbook has 116 pages with 159 + 27 (in chapter 8) citations and 8 illustrations. It is divided into 9 chapters, 38 subchapters, and many topics following each other in logical sequence.

The first is the Objectives discussing here the difficulties of this subject too. Besides this, almost all chapters have an introduction which is very useful for the right understanding and evaluation of the methods described in them.

The impartial criticism based on an excellent professional practice of the editors, features the descriptions of methods (e.g. soil sterilization, the uses and limitations, advantages and handicaps of steam, methyl bromide sterilization, gamma irradiation), leaving the choice to the readers.

The second chapter, the Habitat, gives a detailed description on the selection of site, sampling areas and environmental factors (soil, biotic and meteorological ones).

Sampling, the most important basic procedure, also has a separate chapter on the collection of samples from the field, the sampling units, statistical procedures and general principles of sampling.

For more information on the form, location and growth pattern of microorganisms, microscopic techniques are described in the chapter on Determination of the form and arrangement of microorganisms in the soil: direct examination of soil particles (light- and electron microscopy) and the soil profile; soil sectioning (technique for soil and leaf litter);contact methods (buried;slide technique and impression technique); examination of root-microbe interactions (the Faraeus slide technique and root-observation boxes).

Under the heading of Isolation of microorganisms there is a description of the direct methods (for fungi and bacteria and other direct methods of isolation), further the indirect methods of isolation (planting of untreated and pretreated samples).

Very exciting problems are discussed in the following chapters from production-biological, ecological and soil-microbiological points of view, namely the biomass measurements: expression of biomass (conversion of cell and spore counts, and mycelial lengths, as well as the problems in the application of techniques for soil biomass determinations);

the subchapter on measurement and counting contains a description of the agar film technique, stained smears, dilution- plate count, extinction-dilution method; partial sterilization—reinoculation method and the less known chemical estimations are also recorded here.

The seventh chapter deals with a question for which a better solution is greatly desired, not only by soil microbiologists, that is the determination of microbial activity in the soil. Here the methods known by trained microbiologists are listed and their faults and insufficiencies are pointed out: the measurement of metabolic activity (field and laboratory methods for measuring gas exchange, enzyme-assay methods, thermal measurements etc.); measurements of growth rates (in sterile soil and in natural environment, as well as the determination of competitive saprophytic ability). The subchapter on the growth of microorganisms in model systems contains a concentrated description of soil sterilization, other particulate media, artificial roots and continuous culture techniques and finally the inferential assessment of activity.

In the chapter Identification of soil organisms a bibliography is given for further information on techniques and diagnostic procedures prepared for fungi, bacteria and actinomycetes and from a general point of view.

The last chapter is the Media for isolation of soil microorganisms. Here not only the solidifying and selective agents are discussed, but the preparation method of soil extract media and description of 24 different media too.

It could be mentioned as faults of the work: the media (9.6) are not written down uniformly; sometimes the authors are quoted and at other times not (9.6.1): in other cases the pH, the kind and amount of water, the per cent of agar in the medium or even the time and pressure (9.6.2), etc. are not indicated; or the heading of chapter eight is Isolation of soil organisms when only data concerning microorganisms are mentioned in it. But, of course, these do not decrease the value of the book.

However, in contrast to this, the restricted size of the work is not to its advantage, for the descriptions of the methods are sometimes too brief. It is hard to understand why this is only a half or nearly a third of the size of other handbooks sponsored by the IBP so far. Although references are given at the end, more detailed descriptions would be most desirable giving more information and guidance to the specialists, especially the younger ones.

A fault which, however, cannot be attributed to the editors but to the present developmental stage of soil microbiology is the inadequacy of the described methods (e.g. microbiological activity of soils, etc.).

As it is remarked by the authors too: the soil microbiologists are not yet in a position to answer the questions regarding the rate of microbial cell production in the soil and on the relative rates of metabolic activity of different components of the soil microflora in soil microhabitats.

In spite of the briefness of the handbook, the rapid and enormous development of soil ecology (and as regards the future, hoping this for soil microbiological methods too) we would evaluate this work in accordance with the foreword of the writer E. B. Worthington:

"It will be used not only during the remaining years of IBP but doubtless for a considerable period thereafter."

M. KECSKÉS

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Iraq

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College of Agriculture,
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Abu-Ghraib,
Iraq

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Department of Animal Science,
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Nigeria

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Hungary

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Hungary

Mahmoud S. A. Z.

Department of Microbiology,
Faculty of Agriculture,
Ain Shams University,
Cairo,
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Marczal G.

SOTE Gyógynövény- és Drogismereti
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Pál A.

ATE Fizika-Matematika Tanszéke,
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"K. Malkov" Agricultural
Experimental Station,
Sadovo, Plovdiv district,
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Pozsár B. I.

Agrobotanikai Intézet,
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MTA Mezőgazdasági Kutató Intézete,

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Rod J.

Institute of Agrotechniques,
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Czechoslovakia

Ruch F.

OPTON Feintechnik Gmbh,
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B. R. D.

Sarin M. N.

Cummings Laboratory,
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New Delhi-12,
India

Sinkovics M.

1016 Budapest,
Szirtes út 4/b,
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Sircar S. M.

Department of Botany,
University of Calcutta,
35 Ballygunge Circular Road,
Calcutta-19,
India

Solymosy F.

MTA Biológiai Központja,
6701 Szeged,
Postafiók 521,
Hungary

Sprague G. F.

Department of Agronomy,
University of Illinois,
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USA

Srivastava A. K.

Department of Botany,
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Stankov I.

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Stefanovits P.

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Hungary

Szabó L. Gy.

Kutató Állomás,
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Hungary

Taha I.

Department of Microbiology,
Faculty of Agriculture,
Alashar University,
Cairo,
Egypt

Takács T.

Erdészeti és Faipari Egyetem,
Termőhelyismerettani Tanszék,
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Postafiók 132,
Hungary

Trapp L.

OPTON Feintechnik Gmbh,
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Postfach 35/36,
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Uprety D. C.
Cummings Laboratory,
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India

Varga M.

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Hungary

Venturi G.

Istituto di Agronomia Generale
Facolta di Agraria,
Universita di Bologna,
Bologna,
Italy

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TTM Növénytár,
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Vajdahunyadvár,
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SOTE Gyógynövény- és Drogismereti

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